PRESERVATION of AGRICULTURAL SPECIMENS in PLASTICS

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Preservation of Agricultural Specimens in Plastics

By G. R. Fessenden, formerly *specialist in agricultural specimen preservation, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration*

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INTRODUCTION

IN THE WORK carried out by the United States Department of Agriculture on the development of improved methods for preparing specimens of agricultural material, it was found that the procedures outlined below are practicable within certain limitations for the classes of material defined for each.

The two preservation methods described in this manual are:

1. Preservation of plant specimens by chemical treatment to retain their natural color and, after dehydration, mounting them in two-dimensional form in plastic plates. This method is applicable, with suitable modifications, to flowers, leaves, roots, and such other plant parts as lend themselves to being mounted in a flattened condition.

2. Embedment of three-dimensional specimens of biological material in blocks of transparent plastic. This method is limited to specimens that can be dehydrated without undesirable change in form or color and are not altered in appearance by the action of the plastic during embedding.

Each of these two preservation methods is designed to produce only the particular type of mount described, and is limited in application to the classes of material specified. In selecting the method to be employed for preserving and mounting a given specimen, it is important, therefore, to make sure that it is capable of being processed in the manner desired and that the resulting mount is of the type that will be suitable for the intended use.

The application of these methods should be undertaken only by persons who have a working knowledge of biology and chemistry, or who are under the guidance or supervision of adequately informed individuals.

It is not practicable in a publication of this kind to warn the readers against all dangers to be anticipated in variations of the processes outlined. General cautions are included in part 2, beginning on page 32 and continuing on pages 33, 34, 36, and 38; others are scattered throughout the manual. Different conditions or the use of different compounds and combinations might introduce additional hazards.

The subject matter contained in this publication is based mainly on the development work on specimen preservation carried out in the United States Department of Agriculture. Consideration has, however, also been given to the published results of other workers in the field, and the publications consulted are listed at the end of this manual.

In the development of the methods here described cooperation was received from numerous agencies and individuals, including the Bureau of Plant Industry, Soils, and Agricultural Engineering; the Bureau of Entomology and Plant Quarantine; the Forest Service; the Extension Service; the National Herbarium and other divisions of the Department of Biology of the Smithsonian Institution; the National Bureau of Standards; the botany departments of the George Washington University and the University of Pennsylvania; the New York Botanical Garden; the Brooklyn Botanic Garden; the Philadelphia Academy of Natural Sciences; the Arnold Arboretum and the Gray Herbarium of Harvard University; the Wildflower Preservation Society; and the New England Herb Society.

During the compilation of these instructions the subject matter was

presented in preliminary form in a series of short courses in specimen preservation, held during the first 7 months of 1945 through arrangements made by the U. S. Department of Agriculture Extension Service at the State Agricultural Colleges of Florida, New Mexico, Oregon, Washington, Idaho, Montana, North Dakota (in conjunction with South Dakota), Illinois, and Massachusetts (in conjunction with Vermont, New Hampshire, Rhode Island, and Maine). Members of the college faculties and of the Experiment Station and Extension Service staffs of these States cooperated in amplifying and revising parts of the original text. Acknowledgement is also made of the assistance received from numerous other sources both within and outside of the United States Department of Agriculture.

Part 1

Preservation of Natural Color in Plant Specimens Mounted in Plastic Plates

The process (*25*)[1] described in part 1, which includes fixing of natural colors[2] and mounting of pressed specimens in plastic plates, is adapted especially to plant foliage, sections, and flowers, preserved specimens of which are shown in plate 1.

EQUIPMENT AND SUPPLIES

The essential items of equipment and the principal supplies needed for the preparation of specimens by this method are listed below. The quantities indicated are based on the initial needs of one or two workers and should be increased proportionately when additional persons are to undertake the work. Electrical equipment, including lights and switches, used in rooms where flammable substances or their vapors may accumulate should be of the type approved under Article 500 of the National Electric Code.

Equipment

[Items followed by an asterisk (*) are not essential, but will be found helpful, especially when the work is done on an extensive scale.]

Description	*Number*
Electric refrigerator (large or medium size)	1.
Electric hot plate (adjustable temperature)*	1.
Heated mounting table, comprising an electrically heated water bath, or equivalent, covered with a smooth metal or glass plate, approximately 18 inches by 22 inches (thermostatic control not necessary)	1.
Electrically heated squeegee (small photoprint sealer or equivalent)	1.
Thermometer, 150° C	1.
Laboratory balance, 0.1 gm. to 1,500 gm.*	1.
Hydrogen-ion comparator, pH range approximately 2.5 to 9.0*	1.
Rectangular glass or enamelware dishes with covers, 3 inches by 7 inches by 3 inches, or larger	15.
Glass (or plastic) grids or strips to serve as hold-downs in the rectangular dishes	15.
Glass funnels (or thistle tubes) approximately 1½ inches in diameter	20.
Glass jars, 4-ounce, with mouths approximately 1¾ inches in diameter	20.
Glass jars, 12-ounce or 16-ounce	25.
Clip-board, approximately 10 inches by 16 inches, provided with a hinged arm, for use as a layout frame*	1.
Plant press, strap type, with double faced corrugated paperboard ventilating spacers	2.
Trays (plastic, metal, or wood), approximately 12 inches by 18 inches by ¾ inch	20 or more.

[1] Italic numbers in parentheses refer to Literature Cited, p.71.

[2] The basic process for preserving the natural color in plant specimens as described herein is covered by a patent issued to Fessenden (*25*) which stipulates that the United States Government may use the process royalty-free for Government purposes. While all rights under the patent, other than those granted to the United States Government, are retained by the patentee, he has given his consent to the use of the patented process, as well as the modifications described herein, for educational and other nonprofit purposes.

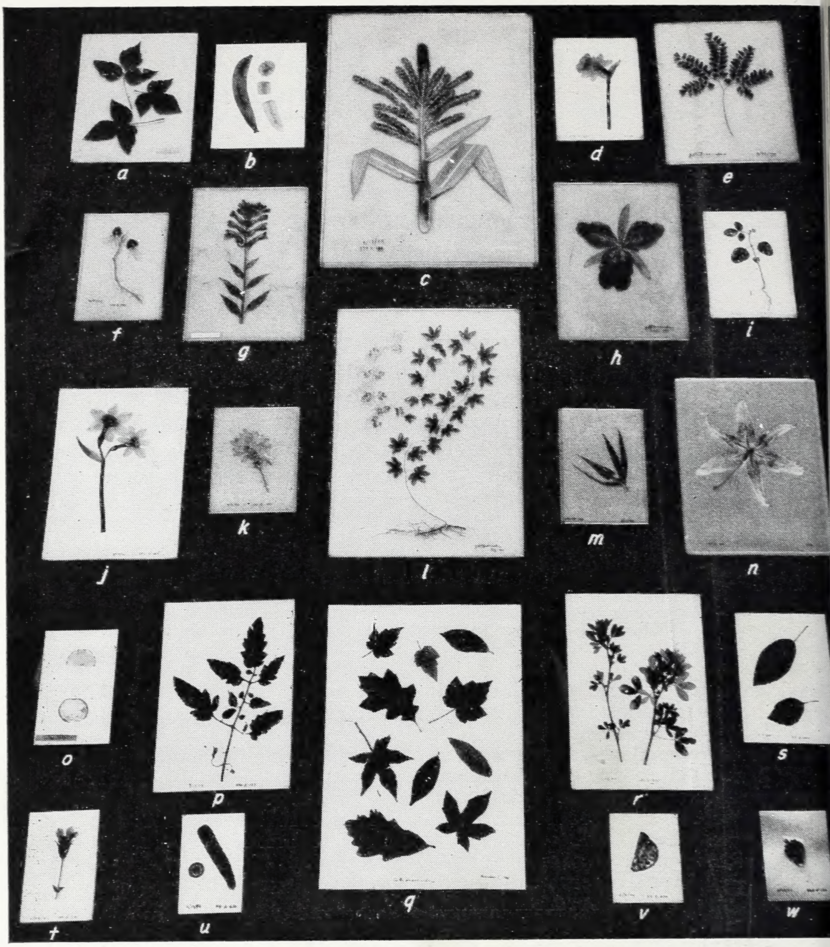

PLATE 1.—Plant specimens preserved in natural color and mounted in plastic plates *a*, Poison ivy leaves in autumn colors; *b*, banana fruit; *c*, corn tassel—staminat flowers; *d*, daffodil; *e*, maidenhair fern; *f*, cockleshell orchid; *g*, cardinal lobelia; *h* orchid flower—*Cattleya* hybrid; *i*, soybean seedling; *j*, narcissus; *k*, guayule leaves; *l* climbing fern; *m*, bird-of-paradise flower; *n*, showy lily; *o*, sections of disease potato; *p*, diseased tomato leaf; *q*, tree leaves in autumn colors; *r*, alfalfa leave showing two stages of leaf-hopper damage; *s*, apple leaves infected with cedar-appl rust; *t*, fringed gentian; *u*, sections of carrot root; *v*, section of tomato fruit; *w*, sectior of strawberry fruit. Specimens were prepared by author using procedures described

Dehydrating boxes or cabinets (metal or wood) large enough to hold 10 or more 12-inch by 18-inch trays. These must be vaportight for use with desiccants	4 or more
Paper trimmer, 18 inches by 18 inches, or larger	1.
Straightedge or ruler, 12 inches or longer	1.
Shears, 8 inches or longer	1.
Wooden (or plastic) forceps or tongs, 6 inches to 10 inches long	2.
Wooden (or plastic) splints (preferably bamboo) 8 inches by 3/16 inch by 1/8 inch	2.

Plastic squeegee, 4½ inches long, tapering from 2½ inches to 1¾ inches wide, 0.03 inch thick	1.
Brush, flat, 1¼ inches wide	2.
Work gloves (canvas or wool)	1 pair.
Biological dissecting set (rust-resistant) including: Scalpels, scissors (straight and curved), forceps, and dissecting needles*	1.
Routine laboratory glassware and implements, including: Beakers, graduated cylinders, measuring pipettes, test tubes, assorted bottles and jars, watch glasses, glass rods, spatulas, stainless-steel knives, and china marking pencils.*	

Supplies

[Trade names are used to identify certain materials that cannot be accurately specified in familiar language. Such use is not intended to be a recommendation of the materials mentioned over other materials that may be found useful for the same purposes.]

Description	*Quantity*
Sorbitol borate nonreducing type preserving fluid, pH 3.5 (designated as SB–1035)	10 pounds.
Sorbitol borate nonreducing type preserving fluid, pH 7.5 (designated as SB–1075)	10 pounds
Sorbitol borate reducing type preserving fluid, pH 5.0 (designated as SB–1150)	10 pounds.
Sorbitol borate reducing type preserving fluid, pH 8.0 (designated as SB–1180)	10 pounds.
Sorbitol citrate nonreducing type preserving fluid, pH 3.5 (designated as SCX–2035)	5 pounds.
Sorbitol citrate nonreducing type preserving fluid, pH 7.5 (designated as SCX–2075)	5 pounds.
Sorbitol citrate reducing type preserving fluid, pH 5.0 (designated as SCX–2150)	5 pounds.
Sorbitol citrate reducing type preserving fluid, pH 7.5 (designated as SCX–2175)	5 pounds.
Sorbitan monolaurate (Span–20)	1 pound.
Sorbitan monolaurate derivative (Tween–20)	1 pound.
Butylphthalyl butyl glycolate (Santicizer–B16)	¼ pound.
Thermoplastic cementing resin (designated as TMR–3011–X)	2 pounds.
Vinylite plastic, clear transparent, in sheet form, press-polished on both sides, size 10 inches by 16 inches, thickness 0.03 inch	40 sheets.
Vinylite plastic, opaque, china white, in sheet form, press-polished on both sides, size 10 inches by 16 inches, thickness 0.01 inch	20 sheets.
Cellulose acetate transparent film:	
Flexible type, 12 inches by 18 inches, thickness 0.0015 inch	100 sheets.
Rigid type, 14 inches by 20 inches, thickness 0.002 inch	60 sheets.
Rigid type, 10 inches by 16 inches, thickness 0.015 inch	4 sheets.
Denatured alcohol (95 percent)	1 quart.
Mineral spirits, painter's grade (petroleum solvent intermediate between gasoline and kerosene)	1 pint.
Waxed paper (household grade)	2 packages.
Distilled water	1 gallon.
Cellulose tape coated with pressure-type adhesive (Scotch tape or equivalent), ½ inch wide	1 roll.
Window glass, double weight, approximately 14 inches by 20 inches	2 pieces.

Some of the supplies listed are flammable. Storage of such materials should be in unbreakable containers, as far as possible. Where more than one person is engaged in the operations, the storage and dispensing of all flammable materials should be the responsibility of one person.

TYPES OF PRESERVING FLUIDS AND THEIR PRINCIPAL CHARACTERISTICS

Because of the differences in the chemical behavior of the differen plant pigments, it has been found necessary to modify the compositio of the preserving fluids to meet the requirements of the various classe of specimens to be treated. Two general types of fluids, consistin primarily of sorbitol borate and sorbitol citrate, have been prepare for natural-color preservation purposes, and the needed modification in pH values, antioxidant properties, and wetting and conditionin action have been worked out for each, in general accordance with different preservation characteristics of the principal classes of plan material. For specimen material in which conflicting preservatio requirements exist because of unusual pigment combinations, furthe experimental modifications may be found necessary in order to obtai satisfactory results.

The principal types of preserving fluids used in this work may b obtained commercially in prepared form, and the intermediate type can be made from them, as required, by mixing together the prope stock fluids in suitable proportions.

The stock fluids specified in the supply list, page 3, carry designation which serve to denote their principal characteristics. The letters preceding the numerical portion of the designation indicate the general type of the fluid; e.g., SB is applied to all types formulated with sorbitol borate, and SCX to all sorbitol citrate types. The first two digits indicate the specific characteristics of the fluid; those in the 1000 and the 2000 series tend to inhibit oxidation but are of a nonreducing nature; those in the 1100 and the 2100 series exert a definite reducing action in addition to their general antioxidant effect. The last two digits in the designations indicate the pH values of the fluids.

Thus the designation SB-1035 means that the nonreducing sorbitol borate type to which it applies has a pH of 3.5, while SB–1075, for example, is a fluid of similar composition but has its pH value adjusted to 7.5. If it is necessary to add a surface-active and conditioning agent to a preserving fluid, its presence may be indicated by adding A after the numerical designation; e.g., SB–1035–A.

Preparation of a Graded Series of Preserving Fluids

In order to facilitate satisfactory preservation of color in the differently pigmented specimens that are ordinarily encountered, it is convenient to use a working series of preserving fluids that have pH values graded by steps of 1.0 from pH 3.5 to pH 7.5. These fluids can readily be prepared by mixing together high pH and low pH stock fluids in proportions that will provide the desired intermediate values. If for example type SB–1045 fluid having a pH value of 4.5 is desired, a mixture should be made which contains 75 percent of stock fluid SB–1035 (pH 3.5) and 25 percent of stock fluid SB–1075 (pH 7.5), and consequently has a resultant pH value of practically 4.5. The approximate pH of a mixture prepared in this manner can be calculated as follows:

75 percent of pH 3.5 component	=	2.625
25 percent of pH 7.5 component	=	1.875
Resultant pH value of the mixture	=	4.500

In like manner type SB–1055 fluid can be prepared by making a mixture that consists of 50 percent each of SB–1035 and SB–1075, and type SB–1065 fluid can be made by using 25 percent of SB–1035 and 75 percent of SB–1075.

A full series of the nonreducing fluids with values in 1.0 or preferably 0.5 steps, from pH 3.5 to pH 7.5, is desirable, because these fluids are used for preserving material containing anthocyanin pigments that require close correspondence between their specific pH values and those of the fluids with which they are treated. In the case of the reducing fluids, however, intermediate pH values are not ordinarily needed, because the stock fluids of this type are designed to meet the pH requirements of the principal pigments for which they are intended.

All regular fluids can be converted to the A form by the addition of 0.1 percent of the surface-active conditioning mixture consisting of equal parts of Santicizer B–16 and Tween–20. This A form, e.g., SB–1035–A, is preferable for most specimens, except where dense pubescence is present, because it is more rapid and uniform in its action.

Measurement of Preserving Fluid pH Values

Because of their high solids concentrations the pH values of the preserving fluids can be measured more satisfactorily with a colorimetric comparator than with an electrometric instrument. Comparators of the LaMotte type or of the Taylor or Hellige types are particularly suitable for making these measurements, although pH test papers may be found to be accurate enough in many instances. The hydrogen-ion concentration tends to vary with changes in the amount of water in these fluids, and it is, therefore, advisable when making pH measurements to add sufficient distilled water to the sample of fluid in the comparator test tube to bring it to a uniform solids concentration of 50 percent. Since the solids content of most of the preserving fluids mentioned in this publication is approximately 80 percent, the amount of water needed to bring it down to 50 percent would be 60 percent of the original total weight.

In making these colorimetric determinations of pH a very small amount of fluid should be used for each test because it has to be discarded after the indicator solution has been added to it. Dilutions should be made with distilled water only, and all test tubes or other containers employed for these tests should be given several rinsings with distilled water before re-use.

Containers for Preserving Fluids

Only glass or other nonmetallic containers should be used for the preserving fluids employed in this process. The four types of containers described below have been found to be convenient for the several purposes for which they are needed.

1. **Dispensing containers.**—For storing working quantities of the various types of preserving fluids to be applied to flattened specimens, glass jars of 8-ounce or 16-ounce capacity are satisfactory. Glass-topped preserve jars are especially suitable, but jars with screw caps may be used if the caps are provided with Vinylite liners or with waterproof paraffin-coated card liners.

2. **Immersion dishes.**—For treatment of specimens that require immersion, glass or porcelain dishes provided with covers are preferable, but enamelware dishes may also be used if the enamel is intact and no metal is exposed to contact with the fluid. The minimum size serviceable for general use is 3 inches wide by 7 inches long by 3 inches deep. Considerably larger dishes are needed for specimens prepared for standard herbarium-size mounts. The minimum depth of fluid that can be expected to give satisfactory results for immersion treatment is 2 inches. Glass or plastic hold-down strips or grids are required to keep the specimen material properly submerged during treatment.

3. **Testing jars.**—In order to make selective preliminary tests for determining which types of preserving fluid are most suitable for specimens upon which no data are available, it is advisable to use a set of wide-mouth jars of approximately 4-ounce capacity which have screw caps lined with Vinylite or paraffined card. These jars should be provided with hold-downs for keeping the specimen material submerged during testing. Small glass funnels or thistle tubes used in an inverted position are especially suitable for this purpose.

4. **Bulk-storage jars.**—For storing reserve supplies of preserving fluids glass jars of 2-quart capacity or larger are convenient. Glass tops or properly lined screw caps should be used.

All containers should be kept closed except when the fluids are being used, because the fluids take up moisture from the air at relative humidities above 50 percent and lose water by evaporation when the relative humidity is below that level. Labels applied on preserving fluid containers should be securely attached, and should preferably be protected with moisture-resistant lacquer or with cellulose adhesive tape.

COLLECTION, STORAGE, AND PRELIMINARY PREPARATION OF SPECIMENS

In handling the specimen material that is to be treated for natural-color preservation care must be exercised to avoid bruising or otherwise damaging it and to prevent its becoming wilted or dried out before the processing is started. Failure to observe these precautions will also tend to lower the quality of the finished mount.

Collecting

In collecting specimens that are to be processed, it is ordinarily advisable to use a vasculum or other suitable tightly closed container which has a moderate amount of moisture in it to prevent the material from drying out before it can be processed or suitably stored. If closed containers are not available, or in the case of large or broad-leaved specimens, damp newspapers will be found serviceable for transporting material after collection, provided they are kept well covered with waxed paper or other suitable wrapping to prevent undue loss of moisture. It is well to wash off as much dirt as possible from the specimens before putting them into the vasculum, and it is often advisable to wrap each specimen individually in waxed paper at the time of collecting. Material should never be crowded in the container, and it is usually best to remove broken or superfluous parts in order to conserve space.

When circumstances permit, specimens should be processed promptly after collecting because the freshness of their condition is generally a factor in the quality of the ultimate results obtained. This is particularly true for plants that lose their petals or leaves easily or which tend to change color while in storage. It is advisable to collect specimens that are to be pressed before application of preservative in a vasculum and keep them turgid until they can be processed under conditions where continuous heating can be maintained as soon as they are put in press.

Storing and Shipping Fresh Specimen Material

Fresh specimens may be stored most satisfactorily in closed containers kept refrigerated at a temperature of 4° to 5° C. (approximately 40° F.). If there is no excess of moisture in the container, specimens are ordinarily not damaged by mold and often keep in a workably fresh condition for a considerable period. Similar containers may be used in shipping fresh plant material to be processed for natural-color preservation. They will prevent damage from drying out or from mold, and if the containers are sealed so that they are vaportight, and the free moisture is kept at a minimum, the material can often be maintained in a suitable condition for a week or even longer without refrigeration.

Preparing Material for Processing

In preparing specimens for processing, it is important first to remove all soil, dust, and other foreign substances adhering to them. In most cases cleaning can be done satisfactorily under a stream of water by using a brush or a moderately stiff feather to help dislodge the dirt. After washing, the specimens should be left exposed to the air long enough for the surface to become dry, before they are put in press or treated with preserving fluid.

Specimens should be trimmed or pruned, if necessary, before processing, and all undesirable discolored or superfluous parts removed. In most cases it is advantageous to cut away the under portions of thick parts such as roots, bulbs, corms, and woody stems. By doing so the material can be made to remain in position more easily when it is laid out, and possible distortion due to uneven shrinkage of the specimen is minimized. It is advisable to employ only rust-resistant scalpels or stainless-steel knives for sectioning material that is to be treated for color preservation, because the use of ordinary steel or other metal implements may cause discoloration of the tissue through formation of metallic compounds with the tannins and other coloring matter that may be present.

PROCESSING THE SPECIMEN FOR NATURAL COLOR PRESERVATION

To obtain mounted specimens in which the natural color is preserved indefinitely, processing of plant material is carried out in three main steps: (1) Preserving and laying out the specimen, (2) dehydrating it, (3) mounting it between sheets of plastic. Before proceeding with the actual processing, however, it is generally advisable to determine which

TABLE 1.—*Preserving fluids required for stabilizing the principal plant pigments*

Principal types of plant pigments and color-producing substances	Color stabilization requirements	Most suitable fluids to be employed when no other pigments are present	Additional fluids that may be used if copigments require them
Anthocyanins: These pigments are largely responsible for blues, purples, reds, and related shades.	The anthocyanins require pH values ranging from 3.5 to 7.5 for accurate preservation of their various colors. They are bleached by reducing agents and, therefore, must be treated with nonreducing fluids only. They tolerate both borate and citrate types of preserving fluids.	SB–1035[1] (scarlet to red) SB–1045 (crimson to rose) SB–1055 (magenta to purple) SB–1065 (lavender to indigo) SB–1075 (blue)	SCX–2035.[1] SCX–2045. SCX–2055. SCX–2065. SCX–2075.
Anthoxanthins: The pigments in this group consist mainly of flavones, flavonols and their derivatives. They contribute mostly pale orange, yellow, and cream tints.	The flavones and flavonols are discolored by the action of borate preservatives; only citrate-type fluids should be used for their preservation. Their color is not altered by pH changes between 3.5 and 6.5. They are subject to oxidative changes and require reducing type fluids whenever their use is not precluded by the presence of anthocyanins.	SCX–2155[1] SCX–2165 SCX–2135 SCX–2145	SCX–2035[1]. SCX–2045. SCX–2055. SCX–2065.
Tannins and other oxidizable color-producing substances: This group includes substances that are normally colorless, or nearly so, but that produce dark colors when oxidized. They occur extensively in pigmented tissue, and also in many white flowers and green [illegible]	Because of their tendencies to produce dark colors when oxidized, especially at pH values above 7.0, the substances in this group require the use of reducing-type fluids whenever the accompanying pigments permit, and they call for pH values as low as the pigments can tolerate. Either borate- or citrate-type fluids may be used.	SB–1155[1] SCX–2155	SB–1035[1]. SB–1045. SB–1065. SCX–2035. SCX–2045. SCX–2055. SCX–2065.

Carotenoids: These pigments produce most of the yellows and oranges, and certain of the orange reds. They are also present in all green tissue in association with chlorophyll.	The colors of most of the carotenoid pigments are destroyed by oxidation, and they tend to become unstable at pH values appreciably below 7.0. Reducing type fluids with pH values between 6.5 and 7.5 should be used for treating them in all cases where the characteristics of accompanying pigments permit. Either borate or citrate fluids may be used.	SB–1165[1] SB–1175 SCX–2165 SCX–2175	SB–1155[1]. SB–1055. SB–1065. SB–1075. SB–1180. SCX–2155. SCX–2055. SCX–2065. SCX–2075.
Chlorophyll: Accompanied by carotene and other carotenoids, chlorophyll provides all the greens and greenish shades in plant tissue.	Of all plant pigments chlorophyll is the most difficult to preserve in its natural state. Its stability is greatest at pH 7.0 to 8.0, but it will in many cases tolerate preserving fluid values as low as 5.5. It is rapidly destroyed by oxidation, and should be protected by the use of reducing type fluids whenever the requirements of accompanying pigments permit. Either borate or citrate fluids may be used for its preservation.	SB–1175[1] SB–1180 SCX–2175	SB–1155[1]. SB–1165. SB–1055. SB–1065. SB–1075. SCX–2155. SCX–2165. SCX–2055. SCX–2065. SCX–2075.

[1] Only the unmodified fluids are listed in this table, but those containing surface-active and conditioning agents (designated by "—A" in accordance with instructions on page 4) may be used interchangeably with them in all cases except where specimens have special surface characteristics, such as dense pubescence, which are changed in appearance by the wetting action of surface-active agents.

groups of pigments are present in the material and to select the types of preserving fluids that appear to be most nearly suitable for treating them.

Determination of Pigment Preservation Requirements

The type of fluid required for preserving the natural color of a given specimen can often be determined with workable accuracy by estimating which pigments are probably present, and then selecting the fluid that has shown itself to be most nearly suited to the requirements of these pigments. With a little experience this selection can usually be made without difficulty by referring to the accompanying tabulation on plant pigments and to the data on preserving-fluid requirements given in table 1. When these data prove to be inadequate for a particular specimen, its preservation requirements can usually be worked out satisfactorily by applying the simple preliminary tests outlined under the heading, "Preservation Requirement Tests," page 12.

Tissue color	*Pigments probably present*
Blue Purple Magenta Rose Red Scarlet	These colors and their usual variations are commonly produced by anthocyanin pigmentation, except in the case of the carotenoid reds and scarlets. Where complex variations involving the colors in this group occur, anthoxanthins or carotenoids are commonly present as copigments. Tannins and other oxidizable color-producing substances also frequently occur in company with the anthocyanins.
Salmon Coral red Fawn Russet Brown Bronze	The more or less complex coloring represented by this group indicates strong carotenoid or anthoxanthin copigmentation in conjunction with anthocyanins. Tannins also often play a part in these mixed colors.
Ivory Cream Light yellow Pale orange	Where pale colors of this type prevail, the anthoxanthins, which are mainly flavones and flavonols, are in most instances the principal pigments present. Tannins and other oxidizable color-producing substances frequently occur with these pigments.
White	Nearly all white flowers contain tannins or other substances that are colorless in the natural state but tend to become brown or otherwise dark colored when oxidized. In the case of flowers that are not pure white, anthoxanthins or small amounts of carotenoid or anthocyanin pigments may be present.
Yellow Orange Orange red	The stronger yellows and oranges almost invariably indicate the presence of carotenoid pigments, as do also the tomato red and similar orange-red shades. Mixed patterns and special shadings in this color group are commonly due to anthocyanin or anthoxanthin copigmentation. Tannins and other oxidizable color-producing substances are frequently present with these pigments.
Green	All green and greenish shades in plant tissue are produced by chlorophyll accompanied by carotenoids. Where purple or red shades of green occur, anthocyanins are also present. Tannins and other oxidizable color-producing substances are also usually present in green tissue, but appear to be absent in certain types.

The preserving fluid requirements for the principal types of plant pigments are described in greater detail for each of the five pigment groups as follows:

1. The anthocyanin pigments require preserving fluids of the nonreducing type only, because they are bleached to a colorless form when acted upon by reducing agents. In order to insure that these pigments will retain their original colors without change, it is necessary to employ a fluid that has a pH value that corresponds closely to the specific pH requirement of the particular pigment that is present. These requirements vary according to the chemical structure of the pigment, and the specific pH values range from below 3.5 for the scarlets and bright reds to 7.5 for the bluest shades. Either sorbitol borate or sorbitol citrate preserving fluids may be employed for the anthocyanins, but the borate types are usually preferable except where the presence of flavone or flavonol copigments requires the citrate types.

2. The anthoxanthin pigments are subject to oxidative changes, and are, therefore, preserved more satisfactorily by the use of the reducing type fluids. Nonreducing fluids, which are required whenever anthocyanin copigments are also present, will, however, give acceptable results in most instances except where pH values above 6.5 prevail. The optimum pH range for the preservation of the anthoxanthins is between pH 5.5 and pH 6.5 but somewhat lower values may often be employed satisfactorily when the presence of other pigments necessitates their use. Since the flavones and the flavonols, which comprise the greater part of this group of pigments, are discolored by boric compounds, only sorbitol citrate (or other borate-free fluids) should be employed for preserving plant tissue containing them.

3. The tannins and other oxidizable color-producing substances, which are naturally colorless but tend to cause browning or discoloration of tissue during preservation, are treated most advantageously with preserving fluids of the reducing type having pH values as low as the accompanying pigments will permit. In many instances, however, nonreducing fluids with pH values below 6.5 may be used for them if anthocyanin pigments are also present. Where pH values higher than 6.5 are required by the presence of chlorophyll or carotenoid pigments, only a reducing type fluid should be employed in order to minimize the tendency of the tissue to darken during treatment. Either sorbitol borate or sorbitol citrate preserving fluids may be used for the tannins and other oxidizable color-producing substances, although the borates are somewhat more satisfactory except where the presence of flavone or flavonol pigments necessitates use of the citrates.

4. The carotenoid pigments are preserved most satisfactorily by fluids of the reducing type, because oxidation, if allowed to take place in pigments of this class, results in eventual loss of color. When the presence of anthocyanin copigments restricts the choice of preserving fluids to the nonreducing types, acceptable results may still be obtained in many instances where such fluids are used, provided their pH values are not below 5.5. In general, however, it is desirable to employ reducing-type fluids with pH values between 6.5 and 7.5 wherever possible for all tissue in which carotenoid pigments occur. Either sorbitol borate or sorbitol citrate preserving fluids may be employed for these pigments, but the former are usually preferable unless the presence of flavones or flavonols precludes their use.

5. Chlorophyll requires reducing type preserving fluids for best results, but can be preserved more or less satisfactorily with nonreducing fluids in many instances where the presence of anthocyanin pigments requires their use. The optimum pH range for chlorophyll is between 7.0 and 8.0, but acceptable results are often obtainable with fluids having measured pH values as low as 5.5, in cases where their use is required because of the presence of anthocyanin pigments or tannins.

Figure 1.—Preliminary tests with representative color-preserving fluids may be required to determine the stabilization treatment needed for each type of plant material. Wooden or plastic forceps are used to handle the samples.

Either sorbitol borate or sorbitol citrate preserving fluids are suitable for chlorophyll, but the former are generally more satisfactory where the absence of flavone and flavonol pigments permits their use.

Preservation Requirement Tests

If the preservation requirements of a specimen cannot be suitably determined through use of the tabulation on p. 10 and table 1, the selection of the proper preserving fluid can usually be worked out by applying the tests (fig. 1) outlined below.

Testing jars equipped with hold-downs and covers as described on page 6 are convenient for these tests. Grouping the jars in the order of

the fluid-type designations will help in interpreting the results. While workable results can usually be obtained at room temperature within 10 to 30 hours, more exact indications are obtained if the jars are kept refrigerated for 4 or 5 days. On the other hand, a much quicker but considerably less reliable indication of the action of the fluids can be obtained in 20 to 30 minutes by raising the temperature of the jars to between 70° and 80° C. (approximately 170° F.). The pigment responses under such conditions, however, often do not correspond in all respects with those obtained at room temperature or under refrigeration and may consequently give misleading indications unless due allowance is made for possible variations.

The types of fluids ordinarily needed for making these tests are listed below. Where the indications obtained with these standard fluids are not entirely satisfactory, other intermediate types should be tried in order to obtain closer correspondence with the pH requirements and other characteristics of exceptional specimen material.

The standard test fluids commonly employed are: Sorbitol borate types SB–1035–A, SB–1045–A, SB–1055–A, SB–1065–A, SB–1075–A, SB–1155–A, SB–1165–A, SB–1180 and SB–1180–A; and sorbitol citrate types SCX–2035–A, SCX–2045–A, SCX–2055–A, SCX–2065–A, SCX–2075–A, SCX–2155–A, SCX–2165–A and SCX–2175–A.

Extra specimen material similar to that which is to be preserved should be used in making tests for selecting the preserving fluid, and the parts to be tested should be cut into as many pieces as will be necessary to provide at least one piece for each jar to be used in the test. In putting the pieces into the jars it is important to make sure that each piece is completely wetted by the fluid and that it is kept submerged well below the surface during the entire period of the test.

After the test pieces have been immersed long enough to make sure that no further changes in appearance will occur, they should be removed and, after most of the fluid adhering to them has been allowed to drain back into the jars, they should be laid out on sheets of thin cellulose acetate film (0.0015 or 0.002 inch thick) and covered with similar film. The excess fluid should then be forced away from the pieces by lightly applied finger pressure, and their appearance then compared with that of the fresh material. Unless time is taken to dehydrate these test pieces, however, allowance should be made for possible further change in appearance that might occur upon drying. After a reasonable amount of experience the action of the preserving fluids can in many cases be judged with workable accuracy while the test pieces are still immersed in the jars.

Since the results obtained with these tests, especially when they are carried out at or below room temperature, are generally similar to those that may be expected when the entire specimen is subjected to preservation treatment, the type of fluid that produces results most nearly in accord with those that are desired should be selected for treating the specimen. When selection of the preserving fluid is made in this way it is not actually necessary to give consideration to the types of pigments present in the specimen, but it will nevertheless be found desirable to note them for the purpose of accumulating information that will be useful in preserving similar material in the future.

PRESERVING AND LAYING OUT THE SPECIMEN

In carrying out this step there are three different procedures to choose from, and selection of the one to be employed depends upon the structure and the preserving characteristics of the specimen material to be processed.

Procedure A can be used only for specimens in which natural color can be retained during pressing and drying. It can be employed for a wide variety of material and is the quickest and simplest to apply, but in many instances the results are not as satisfactory as those obtained with procedure B or C.

Procedure B is primarily for use with material which consists only of thin tissues and which cannot be satisfactorily dried in press or is too complicated in structure to be suitable for laying out after treatment by immersion. It is especially applicable to specimens having unstable pigmentation which possess finely dissected leaves and complex inflorescences; but it may also be used to good advantage in many instances for such material as algae, mosses, and liverworts, leaves of certain conifers, grasses, autumn foliage, and thin sections of fruit and other succulent or woody material.

Procedure C should be employed for material which cannot be pressed satisfactorily and is of a thick or succulent nature which makes it unsuitable for treatment by procedure B. It is also used for specimens that have brittle or fragile parts which cannot be laid out properly before treatment or that have particularly unstable pigmentation which requires treatment with a considerable volume of preserving fluid in order to inhibit color change. Procedure C may also be used, if desired, for various classes of specimens included under procedures A and B, and while it is the most time consuming of the three procedures, it will in many instances yield the best results.

After the appropriate preserving fluid and the most suitable method of applying it have been determined for a given specimen the processing procedure decided upon should be carried out in general accordance with the directions given in the following sections.

It will be found convenient to carry out the hand-processing parts of the work on a sheet of heavy glass approximately 12 by 18 inches in size. Adequate illumination is important, and the use of adjustable lights will be found to be advantageous. Running water for washing hands and implements is highly desirable, and the use of distilled water for rinsing the implements will help in avoiding accidental contamination of one preserving fluid by another. Cloth or preferably paper towels are needed throughout the work.

Procedure A—Drying Specimens Before Applying Preserving Fluid

Preparing specimens for pressing.—Specimens to be preserved by this procedure should after careful cleaning be pressed and dried, preferably in a strap-type plant press provided with corrugated cardboard ventilators and thin blotters and driers. In general, the blotters should be used only when needed to protect the specimens from being marked by the corrugations because they tend to retard the removal of moisture when heat is applied. The specimens should be placed in folders con-

sisting of a single sheet of newsprint or other suitable thin paper, and these folders should be sandwiched between the corrugated ventilators, with or without blotters, as the case may require.

Specimens should be laid out in the pressing folders as naturally as possible by using a wooden splint or preferably a fairly stiff feather or small brush. Only as much material should be laid out in each folder as can be kept properly in position while closing it and placing it in the press. The upper sheet of the folder should be let down carefully to permit making final rearrangements as the specimen is covered by it. Pads of absorbent cotton or cellulose fiber held between sheets of thin paper or gauze may be used in place of the pressing folder, or in addition to it. Such pads are helpful in equalizing the pressure on parts of specimens of uneven thickness. The press should be closed with care to avoid shifting the ventilators, and the straps should be drawn up alternately by moderate stages until they exert the fullest pressure obtainable. Further tightening of the straps is usually necessary after a few hours of drying, and in the case of succulent or bulky specimens this may have to be repeated several times.

Drying with heat to retain the natural color.—As soon as the press has been closed, it should be placed over a suitable source of heat in such a position that the heated air will rise through the corrugations and carry away the liberated moisture as rapidly as possible. By maintaining a temperature of 45° to 55° C. (113° to 131° F.) it will be found that most specimen material can be suitably dried with good color retention in 24 hours or even less time. Still more rapid drying and even better color retention may sometimes be obtained when an electric fan is used to force heated air through the press.

Storing pressed specimens.—If the dried specimens are not to be used at once, it is advisable to transfer them to a storage press which should be kept in a drying case supplied with a desiccant. If this is not done, the colors which have been temporarily retained during pressing and drying will tend to fade in a short time when moisture is reabsorbed from the air.

Care must be used in handling thoroughly dried specimens because of their extreme brittleness. In the case of dried specimens that are to be treated with preserving fluid it is usually advisable to rehumidify them partially by subjecting them to moderately moist air for an hour or two before laying them out for processing. If the air in the room is not sufficiently humid to soften them quickly, they may be placed in a moist chamber or covered for a few minutes with damp newspapers.

Laying out the specimen and applying the preserving fluid.—A sheet of transparent Vinylite plastic, 0.030 inch in thickness and large enough to leave a free space of at least 1 inch all around the specimen, should be employed as the base of the permanent mounting plate. This sheet should have its back protected with plain paper or tissue, and then be laid on a piece of waxed paper large enough to provide a margin of approximately 2 inches on all four sides. A temporary cover sheet of 0.0015-inch flexible grade cellulose acetate film is also required, and this should be cut to such a size that it will extend one-half inch beyond the edges of the Vinylite mounting sheet when placed on it.

In laying out the pressed specimen, a moderate amount of preserving fluid is first applied on the mounting sheet and then spread over the

greater part of the area the specimen will occupy. The specimen, which has been preferably softened by partial moistening, is then placed in position and a small additional amount of preserving fluid applied over its upper surface (fig. 2).

The cover film is next lowered into place in register with the Vinylite mounting sheet so that the one-half-inch overlap is maintained all around. While this is being done, the specimen should be held in position, or rearranged as needed, by using a wooden splint under the film and by working on it with the fingers above the film. When the cover film has been placed properly in position, the waxed paper extending beyond its edges should be folded in over it so as to provide a marginal

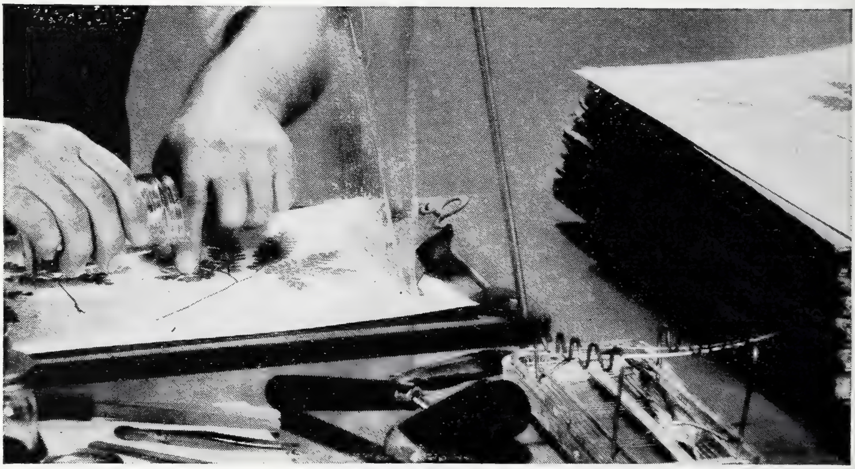

FIGURE 2.—Specimen material that has been pressed and dried before treatment can be laid out on plastic sheets and treated with the preserving fluid.

pocket all around for receiving the excess preserving fluid that is later to be extruded from the layout.

Preliminary working over.—The layout should now be worked over by applying light pressure with the fingers so as to spread the preserving fluid out from the specimen and surround it with a sealed area at least 1 inch wide. This will serve to prevent re-entrance of air when the original bubbles are eliminated. The larger bubbles should then be forced out by displacing them with fluid which is pushed back toward the specimen for the purpose. Finally a moderate amount of fluid should be left in contact with the specimen to provide the necessary preserving action. It is often advisable to allow the layout to stand for an hour or two in this condition before setting it aside to cure, because additional bubbles may form which require elimination.

When the specimen has been worked over as much as needed, the excess fluid around it should be removed by extruding it by means of a plastic squeegee (fig. 3, *A*) or a firm rubber roller (fig. 3, *B*) applied through a free work sheet of rigid type 0.002-inch cellulose acetate film laid over the layout to protect the cover film from being torn or punctured by the extra pressure. The squeegee, or roller, should not be

used over the specimen itself because of the likelihood of crushing or bruising it.

In cases where the preserving fluid has become partially hardened as the result of drying out while standing, it should be remoistened, either by placing the layout in a humidifying chamber for 20 to 30 minutes or by laying wet paper pads on it for a similar length of time.

If the cover film tends to curl or wrinkle at the edges so that it may

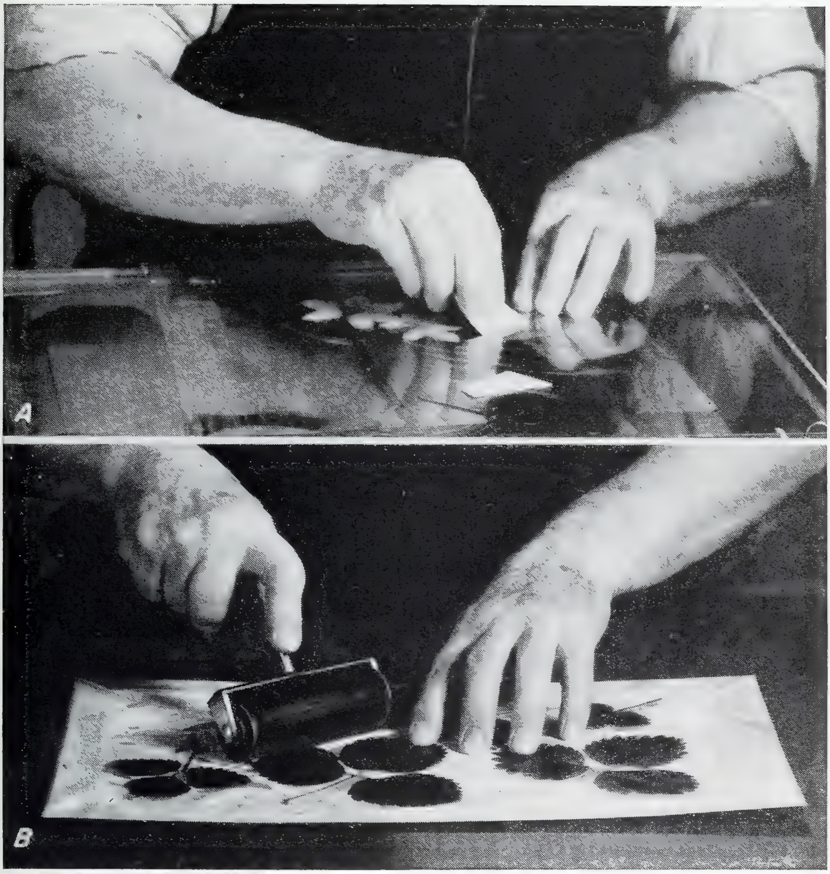

Figure 3.—When the treated specimen has been laid out on the plastic sheet and covered with a cellulose acetate film, the excess of preserving fluid should be forced out with a plastic squeegee (*A*) or a rubber roller (*B*).

admit air to the specimen while being worked over, it can usually be held in place by means of heavy glass or metal strips one-half to one inch wide and of suitable length to cover the marginal area. In case holes that admit air to the specimen develop in the cover film during the working over, they can usually be patched with Scotch cellulose tape; but in the event the holes are too large to patch, the punctured cover film should be replaced with a new one.

Curing specimen material after it has been laid out.—Plant material that has been pressed and dried before treatment, in the manner described in this section, may be cured at room temperatures of 24° C. (approximately 75° F.) or less if the relative humidity is not higher than 60 percent. At higher room temperatures or under more humid conditions, the curing should be carried out under refrigeration at a relative humidity of between 40 and 60 percent. No well defined indications are observable to serve as a guide in determining when specimens processed in this manner are fully cured, but it has been found that a period of 3 to 5 days, depending upon the thickness and the permeability of the specimen, is usually adequate.

At the end of the curing period, the specimen should be examined to make sure that it has remained in proper arrangement and is free from objectionable bubbles. It is not necessary to remove all the bubbles in the preserving fluid around the specimen at this time, because they can generally be taken care of more easily when the protecting cover film is finally removed after dehydration has been completed; but all bubbles directly over or under the specimen should be eliminated before proceeding with the dehydration step. In cases where it is found necessary to rework the layout for the purpose of removing bubbles or rearranging parts of the specimen, the partially dried preserving fluid should first be completely softened again by remoistening the layout under a pad of wet newspapers for at least 20 minutes.

When the specimen has been properly arranged and freed from bubbles, the overlapping waxed paper marginal pockets should be cut off, and the extruded preserving fluid in them may be either reused or discarded. The paper on the back of the Vinylite mounting sheet should now be removed, and the exposed surface of the sheet cleaned with wet paper pads and dried with soft tissue or cloth.

Procedure B—Treating Fresh Specimens With Preserving Fluid Under Film

When the natural colors cannot be satisfactorily retained through the pressing and drying process or when it is difficult to arrange the material in the press, or the material is not suitable for treating in a bath, the processing in most cases can be carried out by this second procedure. This consists in laying out the specimen while it is still in a fresh state and then processing it in the same general manner as under procedure A, except that the curing is carried out under refrigeration.

This procedure is mainly applicable to specimens that are made up of thin parts, and is especially suitable for plants that have finely divided leaves or complex inflorescences or root systems that are difficult to handle either by pressing or by the immersion method. It may, however, be applied if desired to various other types of material in which relatively thin tissues prevail.

Laying out the specimen and applying the preserving fluid.—The specimen should be cleaned thoroughly to make sure that all gritty material that might puncture the cover film is eliminated. The lower mounting plate, which consists of a 0.030-inch thick sheet of transparent Vinylite plastic, large enough to provide a space of at least 1 inch all around the specimen, should be prepared with protective tissue and waxed paper backing as directed for procedure A, and a temporary

cover sheet consisting of 0.0015-inch thick flexible type cellulose acetate film should be cut to a size that will leave an overlap of approximately one-half inch beyond the edges of the Vinylite mounting sheet.

Before laying out the specimen, a moderate amount of preserving fluid of the proper type, as previously determined, is applied to the mounting sheet and spread over the area to be occupied by the specimen. The specimen, which in this case is in a fresh condition, is then placed in position, and a small additional amount of fluid applied over its upper surface. The cover film is next put on in register with the mounting sheet so that it overlaps the required half inch on all sides. The specimen should be kept in its desired position while the cover film is being applied by working under the film with a wooden splint and above it with the fingers. Extreme care is necessary, especially if the tissues are delicate, to guard against tears or bruises which would mar the finished specimen, and pressure should ordinarily not be applied directly upon any but the hard parts of the specimen until the tissues have become toughened through prolonged curing.

The waxed paper is folded over to form pockets for receiving the extruded preserving fluid as explained in procedure A, and the preserving fluid should then be distributed around the specimen by working with the fingers through the cover film until it forms a suitable air seal. The fluid should next be gently worked through the specimen in such a manner that it will displace the larger bubbles and come into full contact with the entire surface area of the specimen.

Curing and reworking specimen material laid out in the fresh state.—Specimens to which preserving fluid has been applied while in a fresh state ordinarily require prolonged curing under refrigeration. As soon as the cover film has been applied and the preserving fluid suitably distributed around the specimen, the layout should be placed in a refrigerator where a temperature of between −4° and 4° C. (approximately 24° to 40° F.) is maintained. The time required for curing at this temperature usually ranges from 10 days to 4 weeks, according to the nature of the material and the temperature and humidity conditions prevailing in the refrigerator. When fully cured, the specimen usually has a characteristically shriveled appearance, and all turgid parts become collapsed; at the same time the tissues develop a definitely toughened condition. Remoistening, through the use of wet paper pads or by confinement in a humidifying chamber, is necessary if the preserving fluid has begun to harden before the layout can be reworked. The procedure for reworking and the use of the plastic squeegee or roller is the same as under procedure A. Upon completion of the reworking, the waxed paper should be removed and the layout cleaned and dried in preparation for the dehydration step.

Procedure C—Treating Fresh Specimens in Preserving Fluid Bath

Fleshy specimens, as well as brittle or easily shattered flowers and leaves and any other material that has been found to be unsuitable for the first two procedures should be immersed while in a fresh state in a bath of preserving fluid..

Succulent fruits, fleshy tubers, bulbs, corms, and similar bulky vegetable material can in many cases be preserved satisfactorily in this way

if they are cut into slices or sections not over 3 millimeters (about one-eight inch) in thickness.

Because the enzymes are inactivated very slowly by this treatment, the best results are obtained by having the preserving fluid chilled to a temperature of 4° C. (about 40° F.) or lower before introducing the specimen material. Suitable glass or plastic hold-downs are required in all cases to keep the material properly submerged during treatment; because, if it is allowed to rise to the top, contact with the air or with the diluted surface layer of preserving fluid may produce a detrimental effect upon both the color and the texture of the tissues.

Immersion of fresh specimen material.—After proper cleaning and other preliminary preparation, specimens should be introduced into the

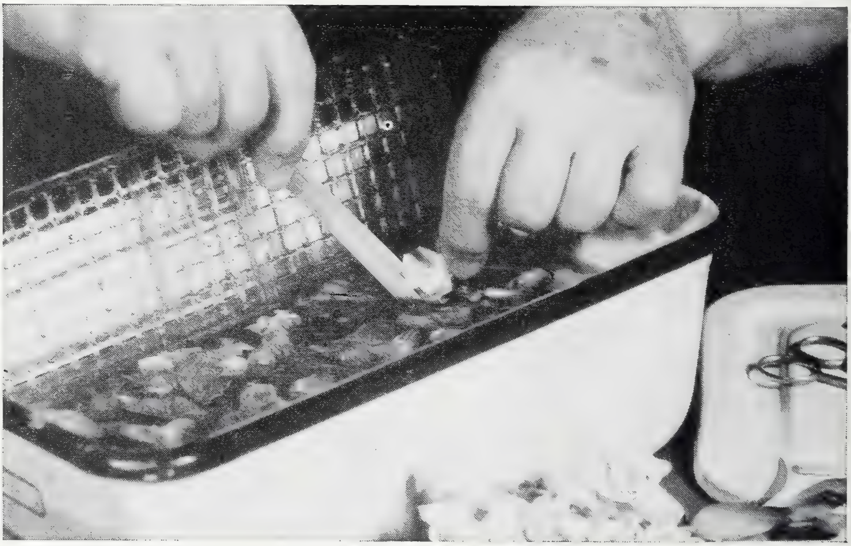

Figure 4.—Fragile or delicate flowers must be introduced into the preserving fluid carefully to avoid injuring them.

appropriate preserving fluid bath in a manner that will insure the complete contact of the preserving fluid with the entire surface area of every part of the specimens, and care must be used to avoid bruising or tearing the delicate tissue (fig. 4). As soon as the specimens are completely wetted by the fluid, the hold-down grids or plates should be placed on top of them to keep them submerged near the bottom of the container where the maximum concentration of preservation is maintained. It is usually advisable to inspect the containers once or twice during the first hour after specimens have been immersed, to make sure that no parts have floated up to the surface or have failed to become wetted by the fluid.

Curing specimens immersed in preserving fluid.—The curing of fresh specimen material in a preserving fluid bath should be carried out at refrigerator temperatures between −4° and 4° C. (approximately 24° and 40° F.) in order to retard enzymatic action which might otherwise

cause discoloration during the interval required for the preservative to diffuse into their tissues. The time required for this curing under refrigeration is usually 10 to 20 days for thin permeable material and may extend to 3 or 4 weeks or even longer for very succulent specimens or those with highly impervious epidermal tissue.

Among the indications that a specimen has become completely cured are: (1) The development of a wrinkled puckery appearance that may be accompanied by considerable shrinkage; (2) increased flexibility except in woody or hard fibrous parts; and (3) noticeable toughening and increased resistance to bruising. If there is any doubt as to whether a specimen has been fully cured, it is advisable to extend the curing time, because specimens removed prematurely from preserving fluid tend to develop discoloration before they can be dehydrated. There is little danger of overcuring the material because it will usually remain unchanged when it is left in the preserving fluid for a number of months, provided it is kept properly refrigerated.

Laying out and working over cured specimens after removal from preserving fluid bath.—When thoroughly cured in the preserving bath, the specimen should be removed with wooden or plastic tongs or forceps and most of the viscous fluid on it permitted to drain off. It is then placed on a mounting sheet, preferably 0.030 inch thick, which is backed with plain paper laid upon a sheet of waxed paper large enough to provide a 2-inch margin all around.

The cured material should be laid out in as natural a position as possible with a wooden splint or similar implement. All folds or creases in leaves and flowers, and all twists or tangles must be straightened out at this time, because these conditions cannot be remedied later. The cover film should now be placed over it in such a way as to keep the specimen in position. This is best accomplished by working with the splint under the film and with the fingers on its outer surface while it is being lowered into position. The waxed paper should next be folded over its edges so as to form a marginal pocket about 1 inch wide all around to receive the excess preserving fluid as it is extruded.

In working over the layout, the air bubbles should be eliminated by pressing out the excess fluid in a manner that will flood the specimen and displace all the free air in and around it. This is done by light finger pressure on the cover film. The excess fluid should then be worked away from the specimen into the surrounding area, and finally extruded into the marginal pockets formed by the folded waxed paper. A plastic squeegee or roller, operated through an interposed work sheet of rigid type cellulose acetate film 0.002 inches thick will be found helpful in pushing the fluid out of the area around the specimen. Pressure should not be applied to the specimen itself with such an instrument. The waxed paper marginal pockets should then be trimmed off and discarded, and after the back of the mounting sheet has been cleaned and wiped dry the layout should be placed in a drying box or cabinet for dehydration.

DEHYDRATION AND PREPARATION FOR MOUNTING

After the preserved specimen has been laid out as described (p. 14), it must be dehydrated to remove all free moisture from it and also from the preserving fluid that still remains around it. This may be done

satisfactorily by placing the specimen layout in a drying box or cabinet in which there is a suitable quantity of granular anhydrous calcium chloride or a similarly effective desiccant (fig. 5). Best results are obtained at room temperature, but moderate heat, up to 55° C. (about 130° F.), may be employed if desired to hasten the final stages. It should be borne in mind, however, that if any appreciable amount of moisture is present in a preserved specimen when it is heated, or if too

FIGURE 5.—Preserved specimens laid out on plastic sheets under temporary cover films of cellulose acetate are dehydrated in a tightly closed box containing a suitable desiccant.

much heat is applied before all free moisture has been removed, discoloration is likely to result.

Three-stage drying.—It will be found convenient to use three drying boxes and to transfer the specimen to each in succession as its dehydration progresses. The first-stage box, where it is allowed to remain 3 or 4 days, will contain partially hydrated desiccant material that has been previously used, first in stage 3 and then in stage 2. Likewise the second-stage box contains desiccant material that has been previously used in stage 3. A slightly longer period for the layout in this box is usually desirable. The third-stage box will contain unused anhydrous desiccant material. The layout should be left in this box until practically all the free moisture has been removed from it. After each lot of layouts has been transferred from one box to the next the desiccant should be moved in the reverse direction and discarded after use in stage 1.

Determining when dehydration is completed.—It is important to make sure in every case that the specimen, and especially the preserving fluid around it, is sufficiently free of moisture to insure satisfactory results in mounting. If any appreciable amount of free moisture is still present in the mounted specimen a whitish cloudiness may develop in the thermoplastic resin around it, and, furthermore, the color of the specimen may tend to change or fade during mounting or subsequently.

A simple way of determining when an adequate degree of dehydration has been attained is to note the hardness of the dried preservative. As moisture is withdrawn the preserving fluid changes to a moderately hard, somewhat brittle, solid. When dehydration has been carried to a point where all of the preservative has reached this solid state the specimen may be considered ready for mounting.

Preparation for mounting.—Before the permanent upper mounting sheet is applied, the temporary cellulose acetate cover sheet that was used to protect the specimen during processing and dehydration has to be removed. This may be done in some cases by stripping it off very carefully after dehydration has been completed. When the film cannot be removed satisfactorily in this manner, it should be moistened with wet paper pads or put in a humidifying chamber just long enough to release it from the dried preservative coating on the specimen. In remoistening for this purpose it is important to apply the humidifying agent for as short a time as possible in order to avoid introducing moisture into the specimen again.

After the temporary cover film has been removed the dried preservative adhering to the Vinylite mounting sheet, except immediately around the specimen, should be removed by using moistened wads of paper toweling or chamois skin and the area then wiped dry with absorbent paper or cloth (fig. 6). It is not necessary to have the Vinylite

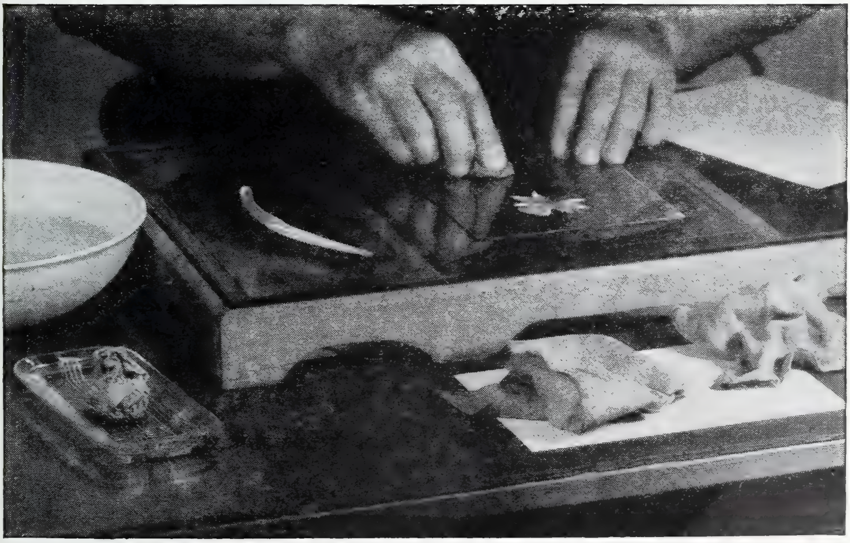

Figure 6.—After the temporary cover film has been removed the dried preservative is carefully washed from the plastic plate with paper toweling or chamois skin moistened with water.

mounting sheet entirely free from paper fibers and dust particles, because the thermoplastic cementing resin used for attaching the upper mounting sheet ordinarily renders them invisible.

Since it is important that both the specimen and the preserving fluid covering it be completely dry before application of the upper Vinylite mounting sheet, the layout should be put into the third-stage drying box again after the cleaning operation and left there for at least 10 hours. This final drying may also be done, if preferred, by heating the layout in an oven at about 55° C. (131° F.) for 4 or 5 hours, or longer if necessary.

An alternative mode of preparation is permissible where no air bubbles have formed around the specimen during dehydration. In such layouts the cellulose acetate cover film may be left in place over the specimen permanently and the upper Vinylite mounting sheet applied on top of it. When this procedure is followed, however, it is necessary to remove marginal strips of the cover film so that an area of the lower Vinylite mounting sheet, at least 1 inch in width, is exposed all around the specimen. This insures the sealing of the upper Vinylite sheet to the specimen when the thermoplastic cementing resin is applied during the mounting operation. This may be done by scoring the cover film lightly with a sharp scalpel or razor blade and then peeling the strips off, using a straight-edge as a guide. After the marginal strips have been removed the exposed area of the Vinylite sheet should be cleaned with moistened wads of soft paper and then wiped dry as explained above. If this temporary application of moisture causes the edges of the remaining portion of the cover film to curl, they should be held down with glass strips until they have dried sufficiently to remain in place again. After this cleaning, final dehydration in a drying box containing fresh desiccant, or in a moderate-temperature oven, is necessary to make sure that the moisture has all been eliminated again.

Storage of dehydrated specimen layouts.—When the mounting operations are not to be carried out at once, specimen layouts may be kept indefinitely after dehydration, provided they are stored in airtight cases or cabinets that are kept supplied with effective desiccants such as anhydrous calcium chloride. Layouts stored in this manner may be removed from time to time for temporary study or inspection, and under relative humidities of 40 percent or lower may be left exposed to room conditions for several hours without appreciable absorption of moisture and consequent deterioration. At higher relative humidities it is advisable to return them more quickly to their desiccant-containing storage cases.

MOUNTING BETWEEN PLASTIC SHEETS

After the final dehydration, the specimen layout is ready for mounting. The mounting should preferably be done in air having a relative humidity below 50 percent, and care must be exercised throughout to keep moisture out of the mount.

Labeling.—The specimen can be labeled, if desired, by writing with water-proof drawing ink on the lower Vinylite mounting sheet, or placing a typed or hand-lettered paper label between the mounting sheets.

Applying overlay film with lubricant.—The top mounting sheet (Vinylite) is cut to the same size as the lower mounting sheet, and two

pieces of cellulose acetate film (rigid type, 0.002 inch thick) are used as temporary overlay sheets during the cementing operation. These films should be large enough to extend 2 inches beyond the edges of the mounting sheets and thus provide space into which the cementing resin can be extruded. One overlay sheet should be applied to the under surface of the lower Vinylite mounting sheet, and the other to the upper surface of the top mounting sheet. These overlay sheets are temporarily attached by spreading on them a thin coating of the water-

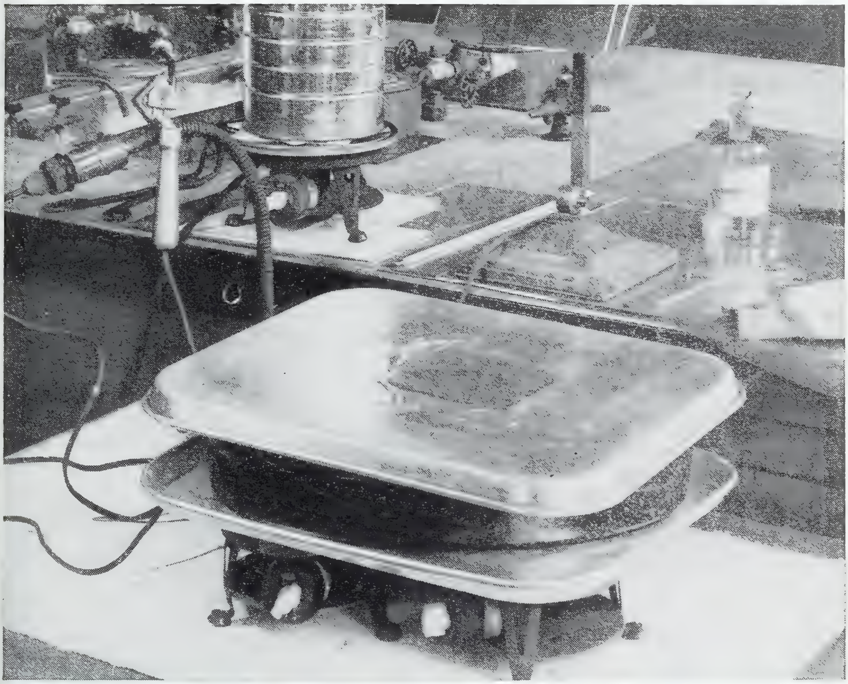

Figure 7.—A heated mounting table can be arranged by placing an inverted metal tray on the lower part of a rectangular or oval roasting pan filled with warm water and supported by another metal tray over a suitable electric hot plate.

soluble lubricant consisting of equal parts of Span 20 and Tween 20. A flat brush or stiff card may be used for applying this lubricant.

Processing the Mount

Applying cementing resin.—The lower mounting sheet, which carries the specimen, is laid on a heated mounting table (fig. 7) which is maintained at a temperature of between 90° and 95° C. (194° and 203° F.). The cementing resin, heated to approximately 110° C. (230° F.) in an air bath should be applied on the face of the mount in sufficient quantity to cover it liberally, the greater amount of the resin being applied over the specimen and smaller quantities near the corners of the mounting sheet.

Preliminary heating.—The mount should now be covered with an inverted tray and allowed to heat for about 5 minutes until the resin becomes workably fluid.

Spreading the resin.—As soon as the resin flows freely it should be spread uniformly over the surface of the mount (fig. 8, *A*) so that no

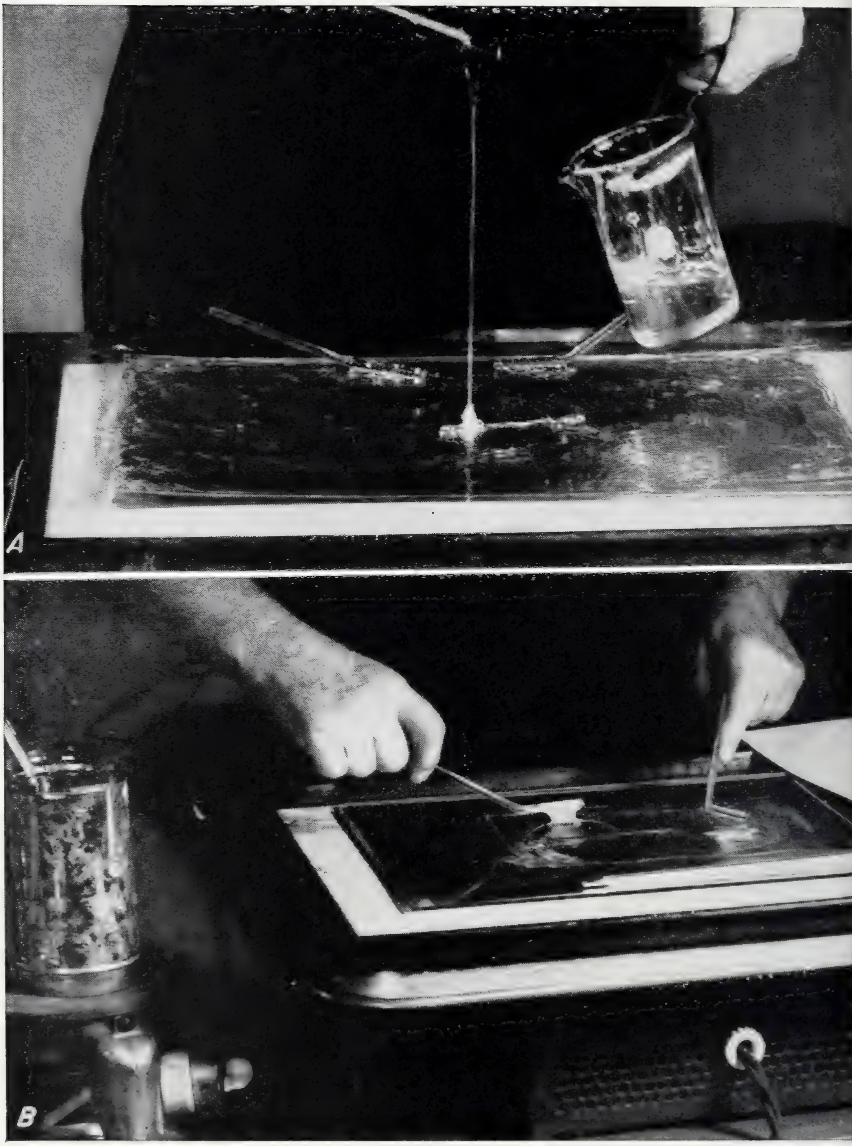

FIGURE 8.—The thermoplastic cementing resin, previously heated to 110° C. (230° F.), is applied while fluid (*A*); it is then spread over the surface of the mount with a glass rod bent like a hockey stick (*B*).

uncovered areas are left. For spreading the resin an 8-inch glass rod bent at an oblique angle 3 inches from the end will be found convenient (fig. 8, *B*).

Applying upper mounting sheet.—The upper mounting sheet with overlay film attached with Span-Tween lubricant should now be applied and kept as nearly in register with the lower sheet as practicable.

Reheating.—The cover tray should be placed once more over the mount and left for 2 or 3 minutes to allow the cementing resin to become workably fluid.

Working out excess resin and air bubbles.—After removing the tray, the mount should first be worked over to eliminate the larger air bubbles. This can best be done with the fingers, which should be protected from the heat by gloves or pads. This preliminary working over should be followed by a careful "ironing" with an electrically heated squeegee—a thermostatically-controlled photoprint sealer is suitable—until all remaining bubbles are worked out and the excess resin has been extruded into the marginal area formed by the over-lay sheets. When using the heated squeegee, a transparent worksheet capable of withstanding the heat satisfactorily should be interposed between the squeegee and the mount to protect the upper surface of the mount. A 10- by 16-inch sheet of 0.015-inch rigid type cellulose acetate film will be found suitable for this purpose.

In working out the bubbles with the heated squeegee, the cementing resin should first be pushed in around the specimen and the excess forced out into the marginal area so as to carry the bubbles with it. Finally, starting on the right-hand side of the mount, "iron" out as much of the remaining resin as can readily be removed. Continue to do so all around the mount until its upper surface becomes smooth and level everywhere except over the specimen. While this is being done, the mounting sheets and the overlay sheets should be kept in approximate register by holding them in position with the left hand.

Cooling and flattening.—After the ironing operation has been completed, the mount is removed from the mounting table and placed on a flat surface where it is allowed to remain a few minutes until it has cooled. During the cooling period it should be held down until it becomes rigid again in order to prevent it from curling. This can be done by weighting it down with a sand bag large enough to cover it, or by placing upon it a stack of newspapers half an inch or more thick and applying enough pressure with the hands or a weight to keep the mount flat while cooling.

Finishing the Mount

Trimming.—After the finished mount has cooled, it should be squared on a paper trimmer. While this is being done the overlapping edges of the overlay sheet should also be cut away together with the excess mounting resin that has been extruded into them. These may be discarded or the resin may be reclaimed by heating the trimmed pieces in a suitable container at 120° C. (approximately 250° F.).

Removing overlay film and cleaning the mount.—After the mount has been trimmed, the overlay film should be stripped off and the mount rinsed in cool water until all of the lubricant has been washed away. In case the cementing resin may have run over the back or the front of

the mount it should be completely removed by using soft paper pads saturated with a cleaning fluid mixture made up of 80 parts 95-percent ethyl alcohol and 20 parts mineral spirits (painter's grade). A final washing with soapy water is advisable to insure removal of finger marks and traces of dirt.

Finishing and applying background.—The edges of the mount should be sandpapered or scraped to make them smooth and properly rounded, and except when a background is to be attached a binding of transparent cellulose adhesive tape should be applied all around in order to confine any excess of the cementing resin that may have been left between the mounting sheets. If a background is desired a sheet of white opaque Vinylite (0.01 inch or thicker) may be attached with adhesive tape in such a way as to bind it all around or to hinge it from the top so that the specimen can be viewed from front or back (fig. 9).

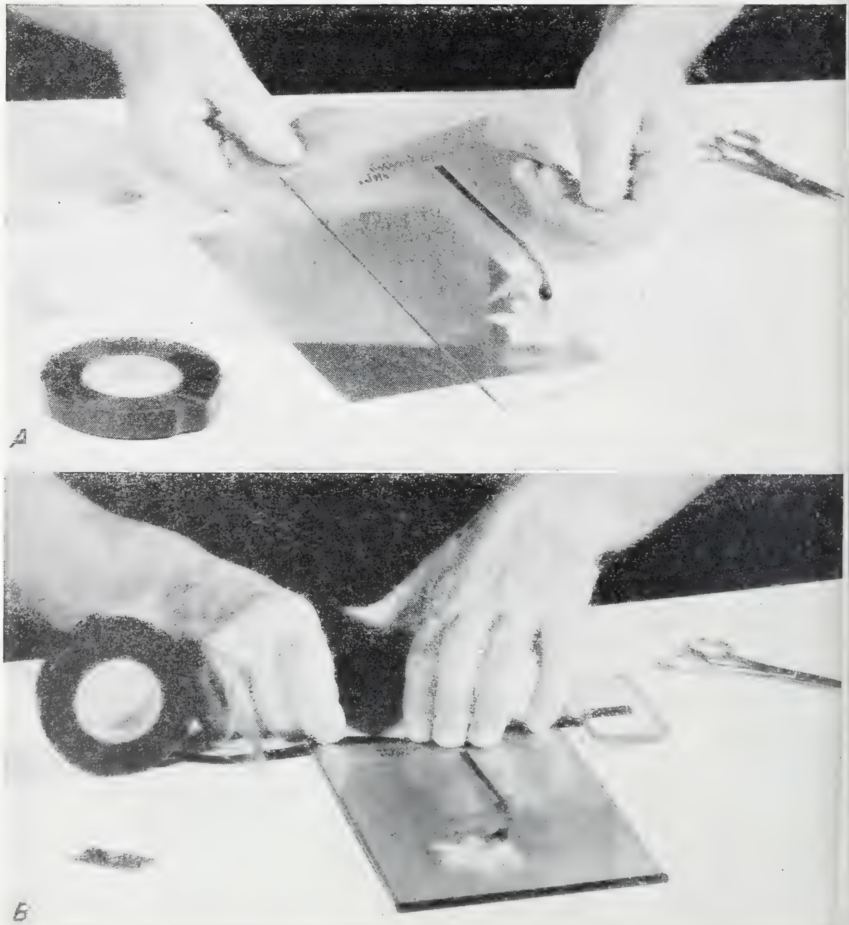

FIGURE 9.—The finished mount is usually provided with a background of metal-coated card or white opaque plastic, overlaid if desired with creped film (*A*). The assembly is bound together with tape coated with pressure-type adhesive (*B*).

CARE OF FINISHED MOUNTS

Natural-color preserved specimens mounted between sheets of Vinylite plastic have been found to be generally resistant to deterioration under normal conditions of use and storage. To obtain the best results, however, it is advisable to observe the following precautions in handling them.

Moisture.—These mounts can be safely washed with water and exposed to extremely humid conditions for moderately long periods. Prolonged or continuous exposure, however, to relative humidities of over 85 percent is not recommended, and where such conditions do prevail, it is advisable to store the mounts in a box or cabinet containing a desiccant.

Temperature.—Temperatures up to 45° C. (113° F.) do not have any apparent effect upon these mounts other than to produce a slight softening of the cementing resin around the edges. In general, however, it is advisable to avoid prolonged exposures to temperatures above 40° C. (104° F.).

Light.—Tests indicate that the colors in specimens that have been properly preserved and mounted in a moisture-free state are stable indefinitely, provided they are not exposed to direct sunlight.

Cleaning.—If these mounts become soiled they should be cleaned with soapy water and then rinsed with clear water. Distilled water is preferable for rinsing because it leaves no residue on drying. Either cold or slightly warm water may be used, but never water hotter than 50° C. (122° F.). Water-insoluble material adhering to the surface should be removed either with ethyl alcohol or mineral spirits or an 80/20 mixture of these solvents. It is not advisable to use any other organic solvents on Vinylite plastic; and ketones, esters, ethers, aromatic hydrocarbons, and chlorinated solvents should in particular be avoided.

Refinishing.—Unless reasonable care is exercised in handling the mounts, abrasions or scratches may in time mar the surface of the plastic. Slight scratches can usually be removed by careful polishing with tripoli polish (or with other suitable polishing compounds) on a buffing wheel. Polishing with magnesium carbonate on wet felt is also helpful in restoring the luster on surfaces that have become dull. All polishing of Vinylite plastic should be done at moderate speeds and with only very light pressure on the buffing wheel in order to avoid damaging the surface of the plastic through overheating.

Storage and shipping.—While no special precautions are necessary in storing or shipping these mounts, it is best to avoid subjecting them to excessive heat and moisture for prolonged periods. Since these mounts are practically no more inflammable than ordinary paper they may be considered as not constituting a fire hazard and can, therefore, be handled or shipped without restrictions. Special safeguards against breakage during shipment are not necessary, but when packing them it is advisable to wrap them individually with cellulose film or with tissue paper or soft fabric as a precaution against abrasions or scratches.

Part 2

Embedment of Biological Specimens in Plastic Blocks

Specimens of biological material that are capable of being dehydrated without undesirable alteration of color or shape, and are not detrimentally affected by the medium in which they are mounted, can be preserved so that their natural appearance is retained indefinitely by embedment in transparent plastic blocks. Plate 2 illustrates the diversity of specimens that can be preserved in this way. In the preservation of specimens by this method, the most satisfactory results have been obtained by employing a plastic-forming embedding medium that can be applied in a fluid state and then be converted through polymerization into a hard, durable solid without the use of pressure or very high temperature.

The procedure for embedding biological specimens in blocks of methacrylate plastic comprises the following principal steps: (1) Removal of inhibitor from monomer; (2) addition of catalyst to monomer; (3) preparation of partially polymerized casting sirup; (4) casting plastic base in mold for specimen; (5) dehydration and preparation of specimen for embedment; (6) embedding specimen and polymerizing plastic around it; (7) heat-treating polymer to prevent subsequent surface crazing; (8) removing cast block containing specimen from mold; (9) machining and polishing finished block.

The essential steps in the embedment of objects in polymerizable resin-forming substances involve the use of certain patented processes (*27, 28, 29, 30, 32*)[3] for which licenses may be required when the work is to be done on a commercial basis. It is therefore recommended that the holders of the patents be consulted in all cases where licensing arrangements appear to be in order.

Various polymerizable substances, including methacrylic esters, phenol-aldehyde and urea-aldehyde condensates, allyl compositions, and alkyd-styrene mixtures, have been reported to be more or less satisfactory for use in this connection. The embedment of organic material in methyl methacrylate, polymerized while surrounding the objects suspended in it, was carried out by Randolph in 1934, and described in his patent (*31*). The applicability of this procedure to the preservation of biological specimens was pointed out by Hibben (*8*) and preliminary results obtained in the embedment of agricultural specimens were reported by Knight (*10*). Preservation of biological material by embedment in phenol-aldehyde and urea-aldehyde resins was described by Brunner and Scheele (*24*). The employment of allyl ester compositions was reported by Brous in 1945 (*5*). Maleic anhydride and styrene mixtures for the embedment of biological specimens were described by Gerhart (*26*). Acrylic vinyl compositions reported to be especially

[3] These patents are all held by E. I. du Pont de Nemours & Co. (Inc.) and limited licenses for their use in connection with specimen embedding may be obtained under suitable circumstances on a royalty-free basis.

suitable for casting and embedding purposes are also described in the literature on plastics (*1*).

In the investigations carried out in the United States Department of Agriculture in this field it was found that methyl and ethyl methacrylates, because of their exceptional clarity and stability in light, were particularly suitable for embedment purposes, and they were accordingly adopted as the basis for the embedding method developed in the Department's research program. Development of this method for

PLATE 2.—Biological specimens preserved in three-dimensional form in plastic blocks. *a*, Seed capsule of *Datura stramonium;* *b*, strawflower; *c*, katydid; *d*, minnow; *e*, closed smut on oats; *f*, yellow and purple pansy; *g*, grasshopper; *h*, mosquito larvae and adults; *i*, blue bachelor's button; *j*, wood tick engorged with blood; *k*, small crab; *l*, dragon flies; *m*, *Penicillium* mold; *n*, Rocky Mountain wood ticks; *o*, ergot sclerotia from grain; *p*, tussock-moth caterpillar; *q*, Japanese beetles; *r*, garden spider; *s*, box-elder bug; *t*, pink rose petals; *u*, screw bean from "tornillo" tree (*Prosopis pubescens*). Specimens were prepared by author using procedures described.

embedding agricultural specimens in methacrylate resins was undertaken in 1937 by C. E. Sando of the United States Department of Agriculture as part of a project for the investigation of processes for preserving specimens of materials related to agriculture in as nearly natural condition as possible. Progress on this work was summarized by Knight in his annual reports (*11, 12, 13, 14*). In 1942 Fessenden and Sando (*6*) published a review of the investigations conducted by other workers in this field and of the procedures and results reported by them.

EQUIPMENT AND SUPPLIES

In order to carry out the embedment of specimens in methacrylate resins safely, it is necessary to employ a fume hood or a similar enclosed space provided with an exhaust fan or other adequate means for removing the vapor and preventing its spread into the room where the work is being performed. This fume hood, or ventilated enclosure, should be large enough to hold two steam or electric ovens, and at the same time leave room for an electric hot plate and the various other pieces of apparatus listed below that are required for preparing the plastic casting sirup. It is also good practice to provide sufficient general ventilation for the work room itself so as to avoid danger of building up undesirable concentrations of the methacrylate vapor that tends to escape from molds, flasks, and other containers.

Enclosed exhaust systems should be arranged to draw air and vapors toward the rear of the enclosure *away* from the operator—*not upward*, since this would not protect the operator. Such an exhaust system should also be arranged to take vapors from the lowest level where heavy vapors may accumulate.

All electrical equipment used in the exhaust system should be of a type approved under Article 500 of the National Electric Code.

Where an adequate exhaust system is not provided, each operator exposed to poisonous vapor should be protected by an air-line gas mask with full face protection supplied by air pumped with approved apparatus from a source known to be safe.

Persons exposed to poisonous liquids or vapors that can be absorbed through the skin should wear rubber or Neoprene gloves.

A 20-pound approved dry-chemical fire extinguisher should be mounted near the exit door.

Conspicuous "no smoking" signs should be exhibited near entrances to and within rooms where the work on preparation of plastic and embedment of specimens in methacrylate plastics is carried on, and the "no smoking" rule should be rigidly enforced.

An electric refrigerator, or its equivalent, is necessary for storage of the monomeric liquid and the partially polymerized sirup. A small or, preferably, medium size, household type of refrigerator is adequate for work by one or two persons.

Two ovens, either steam or electric, are needed for heating the plastic castings during the two stages of polymerization. These ovens, if electrically heated, should be equipped with external contact points and should have enclosed heating elements to minimize the fire hazard. They must also have spring catches to keep the doors closed, rather than

positive latches which might cause explosive rupture of the oven in the event of accidental ignition of the vapor inside.

If the alkali extraction method of inhibitor removal is to be employed, a separatory funnel of at least 1 liter capacity is required.

In case the distillation method of inhibitor removal is preferred, apparatus with all-glass connections is needed. This apparatus should include a three-neck flask of at least 1 liter capacity, a short vertical column packed with glass beads, and distilling head with a 0° to 150° C. thermometer having a standard taper joint, a water-jacketed condenser, and a receiving flask with two or three necks according to whether distillation is to be carried out at atmospheric pressure or under vacuum. A retort heater or heating mantle should preferably be employed, although satisfactory results may be obtained by using an electric hot plate with a sand bath for heating the retort flask.

In addition to the apparatus and equipment already described the following general items of equipment and supplies will also be required.

Equipment

[Items followed by an asterisk (*) are not essential for small-scale operations]

Description	*Number*
Electric hot plate, with temperature control or three-heat switch	1 or more.
Laboratory balance, 0.01 gm. sensitivity	1.
Thermometers, $-10°$ to $150°$ C	2.
Ring stand, medium or large size with assorted rings	1 or more.
Glass funnels, 4 to 6 inches in diameter	4 or more.
Watch glasses, 4 to 6 inches in diameter	4 or more.
Funnel support, 4-funnel capacity	1 or more.
Beakers, 500 ml. and 1,000 ml	3 each.
Beakers, 30 ml., 50 ml., 100 ml., and 250 ml	10 each.
Graduated cylinders, 100 ml. and 500 ml	2 each.
Pipette, measuring, 2 ml	2.
Pipette, transfer, 25 ml. and 50 ml	2 each.
Erlenmeyer flasks, 500 ml. and 1,000 ml	5 each.
Duplex utility clamp (or equivalent)	2.
Water bath, circular form, 8 inches in diameter	1 or more.
Vacuum desiccator, with glass stopcocks, 8 or 10 inch size	1 or more.
Filter pump, aspirator type	1 or more.
Filtering flask, heavy wall, 500 ml	1 or more.
Rubber tubing, heavy wall, for vacuum connections	5 feet or more.
Rubber or Neoprene gloves for each person handling poisonous substances	1 pair.
Biological dissecting set comprising scissors, scalpels, forceps, and dissecting needles	1 or more.
Miscellaneous laboratory implements, including spatulas, knives, shears, rulers, files, glass cutters, brushes, and china-marking wax pencils	1 set.
Assorted laboratory glassware, including rods, tubing, stopcocks, flat glass, jars, and vials	1 set.
Power-driven disk or belt sander	1.
Power-driven buffing head with 6-inch cloth buffing wheels, 1 stitched, 1 open type	1.
Power saw, 8-inch fine-tooth circular saw, or band saw (jigsaw may also be used)*	1.
Power lathe (metal or wood-turning) 3-inch to 6-inch swing, 8 to 12 inches between centers*	1.
Drill press, power grinder, milling machine, and other power tools advantageous for larger scale operation*	1 or more.

SUPPLIES

Description	*Quantity*
Methyl or ethyl methacrylate monomer (produced by E. I. du Pont de Nemours & Co., Inc., and by Rohm and Haas Co.)	8 lb. or more.
Oxidizing catalyst, which may be any of the following, as preferred: Benzoyl peroxide (preferably with 75 percent inert filler, available under trade name Luperco–A), lauroyl peroxide, or tertiary butyl hydroperoxide	2 oz. or more.
Acetone, anhydrous	4 lb. or more.
Denatured alcohol, 95 percent (95 percent ethyl alcohol preferable)	2 lb.
Toluene (Toluol)	2 lb.
Sodium hydroxide, reagent grade, pellets or sticks	1 lb. or more.
Calcium chloride, anhydrous, granular, porous, 8-mesh	5 lb. or more.
Gelatin	½ lb.
Filter paper, Whatman No. 40 or equivalent, 9 cm. diameter or larger	1 pkg.
Hydrogen-ion indicator papers for pH 7.0 to 9.0 (or indicator solutions for same range)	
Cellophane, regular (not moistureproof)	1 roll.
Tinfoil (medium weight)	5 sq. ft. or more.
Scotch cellulose tape, ¾ inch or 1 inch wide	1 roll.
Assorted stoppers (cork and rubber)	
Sandpaper (wet-or-dry type) assorted grits, 2/0, 4/0, and 7/0, or equivalent	6 sheets of each.
Tripoli polish, or other available polishing compound suitable for acrylic plastics, such as Lucite or Plexiglas	1 stick.
Window glass, 9 x 12 inches, or larger	12 pieces.

The equipment and supply items included in the foregoing lists are intended to meet the average requirements for undertaking the embedment of biological specimens on a limited scale. The quantities are based on the needs of one or two workers and should therefore be increased proportionately where the number of persons engaging in the work is larger.

Some of the substances listed are poisonous; some are flammable; and some are both. Such hazardous substances should be stored in unbreakable containers, so far as possible, in a locked metal cabinet. Where more than one person is engaged in the operations, the storage and dispensing of hazardous substances should be the responsibility of one person.

PROPERTIES OF METHYL AND ETHYL METHACRYLATES

In the monomeric state, the form in which they are produced and supplied for embedding and casting purposes, both methyl methacrylate and ethyl methacrylate are mobile inflammable liquids having low flash points and giving off combustible and somewhat toxic vapors. When subjected to the catalytic action of free oxygen, to heat, or to ultraviolet radiation, these liquids are converted by polymerization into clear, colorless, plastic solids which are very stable and nonvolatile at all temperatures ordinarily encountered.

When polymerization of the liquid takes place a shrinkage of approximately 20 percent in volume occurs with corresponding increase in density, and considerable heat is generated as a result of the strongly exothermic character of the reaction. Unless conditions are properly controlled, the heat liberated during polymerization tends to build up high temperatures within the solidifying mass and cause the reaction to

progress too rapidly with consequent formation of vapor bubbles which remain permanently in the final product.

In their solid polymeric form these methacrylate esters are hard, durable plastics that are free from toxic action and have a slow burning rate comparable to that of hard wood or compressed paper. They are resistant to water and to dilute solutions of alkalies and inorganic acids and to their salts. They are, however, attacked by concentrated inorganic reagents and by certain organic acids. They are more or less readily soluble in acetone, ethyl acetate, and various other ketones and esters as well as in toluene (toluol), benzene (benzol), glacial acetic acid, dioxane, chloroform and similar chlorinated solvents, and also in their own monomers. In addition to the active solvents enumerated, various other organic liquids tend to dissolve, soften, or swell them. These plastics are not dissolved or softened by petroleum hydrocarbon solvents such as mineral spirits and kerosene or by mineral oils, nor are they affected by glycerol and other polyhydric alcohols.

Polymethyl and polyethyl methacrylates are entirely stable toward moderate heat and may be raised to temperatures of 200° C. without discoloration or other change. They begin to soften below 100° C. (212° F.) but do not melt. At temperatures much in excess of 300° C. they depolymerize to monomeric vapor which may be recovered in the liquid form by condensation.

These plastic polymers are clear and colorless and have exceptionally high light transmission—approximately 92 percent in the visual range. They are, furthermore, very stable to light under ordinary conditions, and can withstand prolonged exposure to direct sunlight with almost no change.

TABLE 2.—*Principal physical characteristics of methyl and ethyl methacrylates*[1]

Property	Methyl methacrylate		Ethyl methacrylate	
	Monomer	Polymer	Monomer	Polymer
Melting point (° C.)	−48	Does not melt		Does not melt.
Boiling point (° C.)				
760 mm	100.3	Does not boil; depolymerizes to monomer vapor above 300° C.		Does not boil; depolymerizes to monomer vapor above 300° C.
200 mm	61			
100 mm	46			
Specific gravity (at 60°/60° F.)	.950	1.18	0.913	1.11 (at 25°/4°C)
Hardness (Pfund) at 25° C. (grams)		220		141.
Tensile strength (p.s.i.)		9,000		5,000.
Toughness (arbitrary units)		98		174.
Impact strength (Kilogram-centimeters per square centimeter)		10.5		7.1
Refractive index	1.417	1.490	1.414	1.485.

[1] Based mainly on data from Strain, Kennelly, and Dittmar (*18*) and Du Pont de Nemours & Co. (*2*).

Both of these plastics have been found to be suitable for use as embedding mediums for biological specimens. The choice between them depends on only minor differences in their characteristics. Methyl methacrylate polymer is considerably harder and has a higher thermal yield point, but the monomer has a lower boiling point and is more likely to develop vapor bubbles during polymerization. Ethyl methacrylate with its higher boiling point can be polymerized somewhat more satisfactorily, and the polymer is less likely to chip or crack, if dropped, because of its greater toughness; but its impact strength is less, and it has a slightly lower abrasion resistance. A polymer with characteristics intermediate between those of methyl and of ethyl methacrylate may be obtained by mixing the two monomers together in suitable proportions and then polymerizing the mixture.

The characteristics of methyl and ethyl methacrylates that are of particular interest in connection with their use as specimen-embedding mediums are summarized in table 2. Additional data may be found by consulting the publications listed under Selected Bibliography, page 73.

PREPARATION OF PLASTIC

Precautions for Handling Methacrylate Monomer

Because of the inflammability and toxicity of the monomeric methacrylate liquids, special precautions are necessary in handling them. All work with them should be performed in a well ventilated room and as far as possible under a fume hood or similar enclosure provided with an exhaust fan or other effective means for removing the vapor. (See cautions under "Equipment and Supplies," page 32 et seq.) All monomer and partial polymer sirup should be kept in properly closed containers, and spilling should be avoided as much as possible.

Open flames, exposed electrical heating elements, sparking contacts, or other possible means of igniting the liquid or the vapor must never be permitted to come near the work. Ovens used for heating the liquid during polymerization should preferably be steam heated. If electric ovens are used they must have external thermostat contacts, and their doors should be equipped with spring catches instead of positive latches as a safeguard against explosive rupture in case of accidental ignition of vapor inside.

Care must be exercised not to inhale dangerous concentrations of vapor, and it should be borne in mind that both liquid and vapor can be taken into the system by absorption through the skin as well as by inhalation or ingestion. No food or open beverage containers that might absorb the vapor should be put into refrigerators where monomeric or incompletely polymerized methacrylate is kept.

Because of the tendency of the methacrylate monomers to polymerize more or less rapidly at room temperature, they are customarily supplied by the manufacturer in a temporarily stabilized, or inhibited, state. Hydroquinone is the inhibitor generally employed, and before the monomer can be used for embedment, or similar casting purposes where a colorless polymer is required, this inhibitor must be removed as directed in the following section of this publication. It is best, however, to store the monomer in the inhibited state in which it is received until just prior to its use, and as an extra precaution against premature

polymerization, it is good practice to keep it in a place where the temperature is not likely to exceed 20° C. (68° F.).

After the inhibitor has been removed, and especially after the catalyst has been added, it is important to keep all inhibitor-free monomer and partial polymer sirup refrigerated at a temperature of 4° C. (approximately 40° F.) or lower. Whenever chilled monomer or partial polymer sirup is removed from the refrigerator for use during humid weather, it is advisable to allow it to warm up to room temperature before opening the container so as to guard against the entrance of water vapor that tends to condense on the surface as long as it is cold.

Removal of Inhibitor

Before methacrylate monomer can be satisfactorily hardened for embedment purposes, it must be freed of the hydroquinone inhibitor added to it by the manufacturer in order to prevent premature polymerization. This may be done either by distillation, which is the quicker but more hazardous method, or by alkali extraction which is safer and simpler though more time consuming.

Removal of inhibitor by distillation.—The removal of hydroquinone, and similar inhibiting substances, can be accomplished by heating the inhibited monomer in such a manner as to volatilize it, and then condensing the inhibitor-free vapor in a suitable receiving vessel. This should preferably be carried out under vacuum with an arrangement

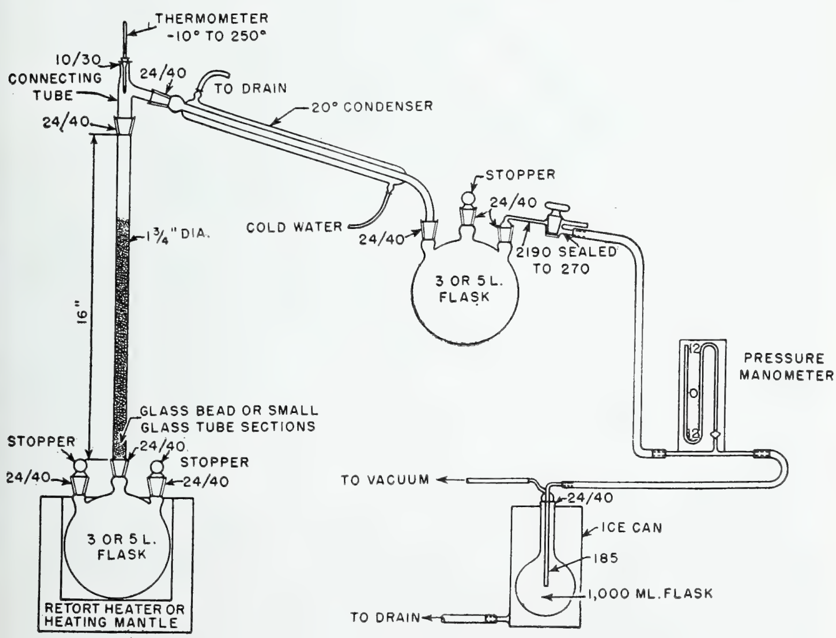

Figure 10.—Arrangement of glass apparatus for vacuum distillation of inhibited methacrylate-monomer liquid. This apparatus should be operated under a fume hood. (Courtesy of E. I. du Pont de Nemours & Co. (Inc.), Plastics Dept.)

of apparatus similar to that shown in figure 10. The apparatus should be used only under a fume hood.

All connections and stoppers used in this set-up should be of glass; rubber and cork generally contain extractable substances which tend to discolor the monomer or interfere with its polymerization. For the same reason the use of stopcock grease and other lubricants, with the exception of those consisting of silicone material, should be avoided for joints exposed to the monomer or its vapor.

For the purpose of heating the monomer to vaporize it, a retort heater or heating mantle as indicated in the diagram is preferable. If one is not available, however, an electric hot plate will serve satisfactorily if a sand or water bath is used with it to distribute the heat evenly over the lower surface of the retort flask. The hot plate should have adjustable heat control and must be of the enclosed element type to avoid undue fire hazard. Gas burners or other open-flame heat sources should never be used in this connection.

If distillation is to be carried out at full atmospheric pressure, the receiving flask must be provided with a relief vent in place of the vacuum connection. This vent should be loosely packed with soft paper or cellophane to allow equalization of pressure without undesirable escape of vapor. When the monomer is distilled under full atmospheric pressure it may show a tendency to polymerize in the retort flask because of the higher temperature required for keeping the liquid boiling under these conditions. This can usually be prevented by putting a small additional quantity of hydroquinone into the retort flask to increase the inhibitor concentration. One-tenth of a gram per liter should be sufficient for this purpose, and it is not necessary to add any in subsequent lots if the residue is left in the flask after the first run.

The general procedure for carrying out the distilling operation follows:

1. After making sure the apparatus is clean and dry throughout, set it up in a fume hood or other enclosure where all escaping vapor will be effectively prevented from diffusing into the room.
2. Pour into the retort flask inhibited monomer until the flask is nearly half full.
3. Start the cold water circulation in the condenser jacket.
4. Turn on the aspirator pump and adjust the flow of water through it to maintain a vacuum of approximately 200 millimeters in the system.
5. Turn on the retort heater and regulate it to keep the monomer in the retort flask boiling gently.
6. Replenish the supply of monomer in the retort flask, preferably just before the flask becomes empty.
7. Empty the receiving flask at sufficiently frequent intervals to prevent overflow into the vacuum line. Pour the distilled monomer from this flask into clean dry bottles, fitted with metal-lined screw-caps, or into flasks stoppered with tinfoil-wrapped corks. Store this inhibitor-free monomer at a reduced temperature of 4° C. (approximately 40° F.) or lower.
8. Take the apparatus apart and allow the monomer to evaporate completely from all joints in order to eliminate the possibility of their "freezing" in place as a result of polymerization. The packed column and the retort flask should be rinsed with a solvent, such as acetone, or toluene to which a little denatured alcohol has been added, in order to remove any partial polymer that may tend to adhere to them.

Removal of inhibitor by alkali extraction.—In removing hydroquinone or similar polymerization inhibitors by alkali extraction, the methacrylate monomer is shaken in a separatory funnel (fig. 11) first with a weak aqueous solution of sodium hydroxide and then with distilled water, after which it is dried over a desiccant to remove any retained wash water.

The procedure for this operation comprises the following steps:

1. After making sure that the glassware is clean and entirely free from grease or other substances that might cause discoloration of the plastic, set up the apparatus in a fume hood or other enclosure where all escaping vapor will be effectively prevented from diffusing into the room. It is

FIGURE 11.—A separatory funnel is used when removing inhibitor by the alkali-extraction method. The operation should be carried out under a fume hood.

advisable to employ enough flasks, beakers, and graduated cylinders so that each can be used for its specific purpose throughout the operation without needing to be cleaned or dried out.

2. Place the separatory funnel in the ring stand and pour into it inhibited monomer until it is slightly less than one-half full. Add an equal volume of 2-percent aqueous sodium hydroxide solution.

3. Remove the separatory funnel from the ring stand, and, while holding its stopper in place, shake it vigorously and intermittently until the two liquids are thoroughly mixed. It is advisable to loosen the stopper once or twice during this operation to release any vapor pressure that may have been built up.

4. Replace the funnel in the ring stand and allow the two liquids to separate by gravity. The lower liquid is the hydroxide solution which is now brownish in color because it contains the hydroquinone. When separation is practically complete, draw off this lower liquid and discard it. While doing so, remove the stopper temporarily from the top of the funnel to admit air for replacing the liquid drawn off.

5. Repeat steps 3 and 4 after adding a fresh quantity of hydroxide solution to the separatory funnel. If the lower liquid now remains colorless it is safe to assume that the hydroquinone has all been removed. If any trace of color appears, however, add hydroxide solution and repeat steps 3 and 4 until the alkali wash is finally colorless.

6. In order to remove the residual alkali, pour into the funnel an amount of distilled water approximately equal in volume to the monomer already in it. Remove the funnel from the stand and shake it vigorously as in step 3 until the liquids are thoroughly mixed.

7. Again place the funnel in the stand, and when separation of the liquids is practically complete, draw off and discard the lower (aqueous) portion.

8. Repeat step 6, using a fresh quantity of distilled water, and this time, after shaking and subsequent separation, draw the discard portion into a beaker that has been well rinsed with distilled water. Test this for alkalinity with litmus paper or with pH indicator solutions or papers having a range above pH 7. If the presence of sodium hydroxide should be indicated by this test, repeat step 6 until a negative test is obtained.

9. Draw all of the monomer from the funnel and transfer it to an Erlenmeyer flask which contains enough anhydrous calcium sulfate or calcium chloride to form a layer approximately one-half inch thick over the bottom. This flask should be tightly stoppered with a tinfoil-wrapped cork and stored at a temperature of 4° C. (approximately 40° F.) or below. The monomer should stand thus over the desiccant for at least 10 hours to make sure all the water has been taken out of it. It may then be transferred to clean, dry storage bottles having metal-lined screw-caps, or it may be left in the desiccating flask until needed. In case this monomer is to be used with a catalyst that does not necessitate filtration, it should be filtered at this time before transfer to the storage bottles so as to remove the particles of desiccant which might otherwise cause haziness in the finished plastic. Continuous storage at the low temperature specified is necessary as a precaution against premature polymerization.

10. If the desiccant used for drying the monomer is of a type that can be reactivated, it should be spread out on a tray after use to permit complete evaporation of the monomer before it is heated for reactivation.

11. Upon completion of the alkali-extraction operation, rinse the separatory funnel and other glassware with water, and allow them to stand in the open air until they are dry and all traces of monomer have evaporated.

Catalyzing the Monomer

In order to facilitate polymerization within the moderate temperature range necessary for satisfactory embedment of most biological specimens, a small amount of an oxidizing catalyst is usually added to the monomer after the inhibitor has been removed.

Among the most suitable catalysts that have been found for this purpose is benzoyl peroxide. Since this is rated as a dangerous fire promoting substance, it should preferably be used in the form of a mixture containing a preponderance of nonoxidizing substances. Such a mixture, which contains 75 percent of inert material, is available under the trade name "Luperco-A". However, since the inert portion of this mixture is more or less insoluble in the monomer, filtration is necessary after the soluble portion has gone into solution.

The amount of benzoyl peroxide that can be employed ranges from 0.05 percent to 0.2 percent on the weight of the monomer, according to the rate of polymerization desired; but in most instances it is advisable to use 0.1 percent or less, to avoid unduly rapid polymerization. When Luperco-A is used, four times the net amount is required, because this mixture contains only 25 percent of active benzoyl peroxide.

In the catalyzing operation, and all the steps that follow, it is important that all glassware be entirely clean and dry. The steps for this operation are as follows:

1. Weigh the amount of catalyst needed for the quantity of monomer to be catalyzed, in accordance with the proportions given above, and place it in an Erlenmeyer flask or other suitable glass container provided with a tinfoil-wrapped stopper.

2. Pour the monomer into the flask and allow it to stand for about 30 minutes. Stir or shake frequently during this time to obtain more rapid solution of the soluble portion of the catalyst mixture.

3. When the active catalyst is all in solution, filter the monomer through fine-mesh filter paper to remove all trace of cloudiness. A paper at least as fine as Whatman No. 40 or its equivalent will be needed to insure complete clarity of the filtrate. Keep both funnel and receiving flask suitably covered to prevent escape of vapor. Several sets of funnels and flasks may be used if desired to cut down the time required for filtering through this fine paper, or vacuum filtration may be employed.

4. Accumulate the clear filtered monomer in bottles provided with tight fitting, metal-lined screw-caps, and store under refrigeration at 4° C. (approximately 40° F.) or below. It should be borne in mind that catalyzed monomer has an active tendency to polymerize at room temperature and should therefore be kept cold at all times except just prior to use.

In place of the crystalline benzoyl peroxide type of catalyst, it is also possible to use a liquid type, such as tertiary butyl hydroperoxide, which is somewhat safer to handle and is completely soluble in the monomer. If tertiary butyl hydroperoxide is employed, however, the

rate of polymerization is usually slower because the available oxygen is released less readily than is the case with benzoyl peroxide. This catalyst is generally applied in the same proportions as the solid catalysts, *i.e.*, between 0.2 and 0.05 percent on weight of the monomer. Except in cases when the monomer contains particles of desiccant or other substances that impair its clarity, no filtration of the catalyzed monomer is needed when a completely soluble liquid catalyst of this type is used.

Preparing Partial Polymer Casting Sirup

For most casting purposes it is desirable. after catalyzing the monomer, to convert it to a partially polymerized sirupy form that is somewhat less volatile than the original free-flowing liquid. This sirup has the further advantage that it requires less time for final polymerization and shrinks less when converted to the solid form than the original liquid. It is best to prepare two grades of this partial polymer casting sirup, one about the consistency of moderately viscous honey, and the other just slightly more viscous than glycerine. The former is to be used principally for casting the supporting base and the layers above the specimen, and the latter for impregnating the specimen and for casting the layers in which it is embedded. An ample supply of unthickened monomer should also be reserved for use in rinsing the specimen and for impregnation in certain instances, and it may furthermore be needed for thinning the partial polymer sirup in cases where it has become too thick for use.

Water-bath method.—Preparation of the partial polymer sirup is preferably carried out by applying moderate heat to the catalyzed monomer by means of a water bath until the desired degree of viscosity has been obtained, and then chilling it thoroughly to arrest further polymerization. The steps required for this operation are as follows:

1. Clean and dry a Pyrex-type glass Erlenmeyer flask (preferably of 1 liter capacity) that is equipped with a vented stopper to permit equalization of pressure without loss of vapor. For this purpose a distilling column may be used or, if preferred, a suitable stopper can be improvised by cutting off the upper tube portion of a 50-ml. transfer pipette and inserting it in inverted position in the neck of the flask (fig. 12). The enlarged part of the pipette should be wrapped with cellophane so that it will fit snugly enough to retain the vapor but at the same tirm be free to release itself in case of sudden building up of internal pressuee. Cork or rubber stoppers should not be used in this connection because of the likelihood of substances being extracted from them that will discolor the monomer or inhibit its polymerization.

2. Pour into the flask to approximately one-third of its capacity catalyzed monomer that has the same temperature as the room. If the monomer is to be taken from a container removed from the refrigerator, it should be allowed to warm up before pouring, in order to avoid the possibility of introducing water into the monomer as a result of condensation of atmospheric moisture. After pouring the monomer into the flask, put the vented stopper in position. As a safeguard against possible entrance of water during heating in the water bath, it is advisable to wrap the joint with a piece of cellophane about 4 by 8

Figure 12.—A transfer pipette of suitable size, with the sucking end cut off and the bulb wrapped with cellophane to fit the flask's neck, provides a vented stopper which condenses the vapor and prevents its escape. Because the flask might break, it is safer to heat it under a fume hood, and to leave it there until contents are cool.

inches and to tie the film at both top and bottom with string or heavy thread.

3. Attach a utility clamp (or its equivalent) to the neck of the flask to serve as a supporting handle and then set it in a water bath on an electric hot plate. A ring stand may be used in connection with the clamp to hold the flask in position during the early stages of the heating, but as soon as the liquid begins to thicken it is better to hold the flask by hand so that it can be more easily manipulated in case the polymerization tends to speed up unduly. A water bath with concen-

tric cover rings and a side steam-vent is especially suitable, but any substantial dish or pan may be used for heating the water which controls the temperature of the monomer during this operation. A large pan or pail filled with ice-water or with running tap-water should also be provided for chilling the monomer in the flask at the end of the operation or in case polymerization tends to proceed too rapidly.

4. Adjust the temperature of the electric hot plate so that the water bath is kept boiling gently, and let the flask remain in the bath for about 5 minutes. At the end of this time—or earlier if any indications of boiling are noted in the monomer—the flask should be removed from the water bath and shaken or swirled in a manner to equalize the distribution of heat in the liquid. The flask should then be replaced in the bath and heated under close watching and with frequent shakings to maintain uniform heat distribution and guard against too rapid progress of the polymerization reaction. In any instance where the boiling does not subside readily or tends to become more violent after shaking, the flask should be immersed promptly in cold water (preferably ice water or running cold water in a large container) and moved about in a manner to chill the sirup as quickly as possible in order to prevent the reaction from getting out of hand.

The partial polymerization should be continued in this manner until the desired viscosity is attained; then the reaction should be completely arrested by chilling the flask for 10 to 20 minutes, or preferably longer, in the cold water. If, after being thoroughly cooled, the sirup shows a tendency to warm up again upon standing at room temperature, further chilling is necessary because this is an indication that the reaction is still going on and, if not checked, will continue at an increasingly rapid rate until polymerization is completed and the plastic becomes a solid mass, which would be useless for embedding purposes.

In judging the viscosity attained before discontinuing the heating, it should be borne in mind that the sirup will become slightly thicker when cooled. If too high a viscosity has developed, it is usually possible to thin the sirup after cooling by adding a small amount of monomer and then shaking or stirring the mixture until it becomes uniform. In any case where the plastic has become solid through complete polymerization, it may be reclaimed slowly by adding a small amount of catalyzed monomer and allowing it to stand for a day or two under refrigeration until the monomer has dissolved enough of the hard polymer to form a sirupy mixture that is usable for casting. As soon as this sirup reaches the desired viscosity, it should be transferred to a separate storage vessel and more catalyzed monomer poured over the hard polymer in the original container to dissolve an additional quantity. This may be repeated until all of the polymer has finally been converted into usable casting sirup.

5. When the polymerizing reaction has been arrested beyond any doubt, the sirup should be transferred to a storage jar having a metal-lined screw cap or to a flask fitted with a cellophane-wrapped stopper.

Tinfoil is not suitable for wrapping stoppers to be used beyond this stage because it tends to become torn as a result of the increased viscosity of the sirup, and shreds of it get into the sirup. Partial polymer should be stored under refrigeration at a temperature of 4° C. (40° F.), or lower, at all times except when it is being used, because it has a tend-

ency to harden rapidly if exposed to room temperature for any length of time.

6. When the partial polymerization has been completed, allow the residual monomer in the vented stopper to evaporate, and if necessary wash away any partial polymer that may have accumulated in it, using acetone or a mixture consisting of 80 parts of toluene and 20 parts of denatured alcohol. The residual sirup in the flask may be left to combine with the new lot of monomer if the operation is to be repeated soon; otherwise it should be thinned with a small amount of monomer and poured into the low viscosity partial polymer storage jar, and the flask then rinsed with acetone or the toluene-alcohol solvent mixture.

Other methods.—Two other procedures may be employed, if desired, for preparing partial polymer sirup. One that requires considerable amount of lapsed time but very little attention consists in preparing, by application of heat or ultraviolet radiation, a small amount of polymer 1 to 2 inches thick in the bottom of a flask or other convenient glass container and then pouring over it catalyzed monomer to a depth of several inches. This is allowed to stand at room temperature until it has become partially polymerized to the desired degree through the "seeding" action of the polymer. During the period of standing, it is well to shake the flask several times a day in order to maintain a fairly uniform consistency throughout the sirup, and it is furthermore advisable to keep the flask stored in the refrigerator except during working hours when it can be inspected with suitable regularity to observe the progress of the polymerization.

The third procedure produces comparatively quick results but requires close attention and considerable manipulation to keep the reaction under control. It is similar to the water-bath method, except that the heat is applied directly to the vessel containing the monomer, and consists in placing the flask directly on an electric hot plate for 3 to 5 minutes and then holding it about an inch above the heated surface until the sirup attains the desired viscosity. The flask should be provided with a vented stopper as explained under water-bath method, and should preferably be not more than one-third filled with monomer. The hot plate should be operated with its switch at the low heat position, and the flask should be kept more or less continuously in motion after the first few minutes, as a precaution against localized overheating.

Whenever any tendency toward excessive boiling is noted, the flask should be removed at once from the heat source and shaken vigorously, and, if necessary, chilled in cold water. When all spontaneous bubbling ceases, heating may be resumed and continued cautiously until partial polymerization is completed. Thorough final chilling is important in order to prevent uncontrolled continuance of the polymerization reaction during storage.

SELECTION OF SPECIMENS

Preservation of biological specimens by embedding them in blocks of plastic formed by polymerization of methacrylate esters is applicable to both plant and animal material, but it is limited to durably pigmented specimens that can be dehydrated satisfactorily and are strong and rigid enough to withstand deformation while the plastic is hardening. All such specimens must, furthermore, be free from extractable substances

that tend to discolor the plastic or inhibit its polymerization, and they must not be subject to changes in appearance due to contact with a surrounding medium having an index of refraction different from that of air.

The types of plant material generally suitable for embedment in methyl or ethyl methacrylate resins include mature grains and seeds, dried fruits, nuts, cones, stems, roots, and sections of wood and bark. A limited number of durable flowers, including strawflowers and other everlastings, and certain types of tough or rigid leaves lend themselves to preservation by this method; but flowers and leaves in general usually prove to be too fragile or too readily affected by the resin to yield satisfactory results.

The zoological specimens that lend themselves to preservation by this method include both adult and immature forms of most of the invertebrates, and also many of the smaller mammals. Insects with rigid integuments give particularly good results, as do also such objects as bones, teeth, horns, claws, carapaces, and shells. Soft-bodied adult insects, as well as larvae and eggs may also be embedded satisfactorily if they can be completely infiltrated with plastic and are strong enough to withstand distortion or rupture during polymerization. Sections of organs and body tissue have been embedded successfully after dehydration by freeze-drying or other special preparation. Hair, fur, and feathers give only moderately satisfactory results, and butterflies, moths, and other objects that owe their coloration mainly to diffraction rather than to pigmentation are generally unsuitable for embedment in plastics of the methacrylate type.

PREPARING SPECIMENS FOR EMBEDMENT

Before the preservation of biological specimens by this method is undertaken their suitability for embedment in methacrylate plastics should be ascertained by reference to the classes of material reported to be satisfactory, which are described under "Selection of Specimens", and, if any doubt exists, by making preliminary trial embedments. It is also advisable to determine as definitely as possible the preparation requirements of the specimen material by reviewing the following procedures, and, if necessary, by carrying out such experimental tests as may seem to be in order for preliminary preservation and dehydration.

Cleaning Specimen Material

Material that is to be embedded should be carefully cleaned to remove all dirt and other undesirable substances. If the specimens are in a dry condition, it is usually advisable to dust them off with a brush or feather, or with a gentle stream of air, and then to wash them by immersion for a few minutes in 95 percent alcohol or dry acetone. After being washed, they must be allowed to stand in the open air until the solvent has evaporated; then they should be stored in a desiccator for a day or more to make sure that all free moisture has been removed. Specimens containing oils or other extractable substances that tend to discolor or modify the plastic during embedment, should, after they are thoroughly dry, be immersed for 24 hours in a solvent mixture composed preferably of 40 parts of toluene, 30 parts of acetone, and 30 parts of absolute

ethyl alcohol (or 99-percent isopropanol), and then rinsed in a second clean bath of the same solvent mixture. In extreme cases, several such immersions and rinsings may be required to remove all the extractable matter that might otherwise have a detrimental effect upon the finished mount. Carbon tetrachloride, chloroform, or dioxane may also be used for this purpose if preferred.

Soft or fleshy specimens that contain considerable amounts of moisture should generally be washed in warm water and rinsed in 50-percent alcohol. They should then be subjected promptly to the first step of the appropriate preservation procedure.

Specimens containing limited amounts of moisture, which are composed of thin tissues and are capable of being air-dried without preliminary preservation, usually require no cleaning other than the removal of dust or other undesirable matter on the surface. This should be done with a soft brush or feather, or with a gentle stream of air, as in the case of dry specimen material.

Preservation and Dehydration

In preparing specimens for embedment in methacrylate resins, the principal aim is to bring them into a stable moisture-free state with minimum alteration of shape and color. Protection from actual contact with the liquid resin is also necessary in certain instances in order to prevent undesirable changes in the appearance of the specimen or in the plastic block during embedment.

Since various classes of specimen material require different procedures for preservation, dehydration, and protective coating, the particular treatment to be employed for a given specimen should be based on the requirements of the class to which it belongs. In applying the general directions given below for the various classes of material, it should be borne in mind that experimentation will often be necessary in order to determine the most effective procedure and, on the other hand, that occasional specimens will be encountered for which no entirely suitable preparatory treatment can be devised.

Rigid specimen material with durable pigmentation.—Mature grains, seeds, sections of wood, everlasting flowers and other sturdy, dry plant material, and also such specimens of animal material as can be satisfactorily dried in the open air without change in appearance, including bones, teeth, claws, shells, and insects with firm integuments, can usually be prepared for embedment by dehydration alone. The necessary dehydration can be carried out conveniently at room temperature in a desiccator, preferably under vacuum, where it should be allowed to remain for at least 24 hours. In the case of specimen material that can withstand moderate heating without change in appearance, drying for 3 or 4 hours in an oven at a temperature of around 70° C. (between 165° and 170° F.) may be employed as a more rapid means of dehydration. If the specimen is not to be used as soon as dehydration is completed, it should be kept in a dry condition until the embedding work is started.

Rigid specimen material with unstable pigmentation.—This class of material includes such specimens as dry plant bulbs, roots, corms, bark, woody fungi and lichens, as well as various objects of animal origin. Because of the rigidity of this material, its dehydration may be

carried out in desiccator or oven as explained in the preceding section. The coloring matter, however, requires protection from actual contact with the resin during embedment in order to prevent either discoloration of the plastic through extraction of soluble pigments or alteration of specimen color as the result of bleaching by the catalyst or modifying action by the resin while in the liquid state.

Protection can in most cases be obtained by coating the specimen with a water-dispersible colloidal substance such as acacia gum (gum arabic), gelatin, egg albumin (egg white), casein, algin, polyvinyl alcohol, methyl cellulose, or carboxymethyl cellulose. Various prepared glues and pastes may also be used. To prevent peeling or cracking of the coating when dry, it is generally advisable to plasticize the coating substance with glycerine, sorbitol, or polyethylene glycol. The amount of plasticizing liquid needed in any given case should be determined experimentally. The coating material should be applied as an aqueous solution (or dispersion) having a moderately low viscosity, and best results can be obtained by applying several successive layers and allowing ample drying time for each. When gelatin or glue is used, treatment with 5- to 10-percent formalin to render each layer insoluble may in some instances prove to be helpful in building up a suitably impervious coating. In the application of protective coatings, the specimens are ordinarily dipped into the solution; but painting or spraying may sometimes prove to be preferable.

Specimens having pigments that do not permit the use of water-dispersible colloidal substances can in some instances be protected more or less satisfactorily by coating them with cellulose acetate, or with a transparent plastic such as vinyl acetate-chloride copolymer dispersed in a suitable organic volatile solvent and applied by dipping or spraying. Several layers are usually necessary, and complete evaporation of solvent from each coat is necessary before the next is put on.

Nonrigid durably pigmented material.—This class comprises two types of material: (1) nonrigid specimens that retain their color satisfactorily when dehydrated in alcohol or in similar moisture-removing volatile liquids, or when subjected to freeze-drying; and (2) plant material made up mainly of thin tissues having low moisture content that can be air-dried without undesirable change in appearance when packed in suitable dry granular dehydrating substances.

The first type in this class includes soft-bodied insects and other zoological specimens that have more or less stable pigmentation, and also a limited variety of fleshy plant material. Specimens of this type usually can be prepared by dehydration in suitable liquids without preliminary preservation. Ethyl alcohol is the most generally employed dehydrating agent for such material, but isopropyl alcohol, tertiary butyl alcohol, acetone, and dioxane can also be used, and in certain cases may yield superior results. Because of the volatility of these liquids, all containers in which they are placed should be kept covered and should not be brought near open flames. In carrying out the dehydration of nonrigid material in water-absorbing volatile liquids it is usually necessary to do so gradually through a number of stages in order to prevent the shrinkage and distortion which tend to occur when the entire water content is removed in one operation.

The first step in dehydration consists of immersing the specimen in a bath of alcohol (or other suitable dehydrating liquid) which has been

diluted with water to a 50-percent concentration. In the case of exceptionally soft or delicate material it may be advisable to start with a concentration as low as 30 percent. The specimen should be allowed to remain in this first dehydration stage for 4 to 24 hours, depending on its size and permeability, until it has become uniformly saturated. It should then be transferred successively through additional alcohol baths having concentrations of 65, 80, and 95 percent, being allowed to remain in each for the same length of time as in the first. When completely permeated with the 95-percent alcohol, it should be transferred into absolute (100-percent) alcohol where it is to be left for 24 hours, or longer if desired, prior to impregating it with methacrylate monomer.

If the dehydrated material is to be stored in absolute alcohol for any length of time, it is advisable to guard against the use of alcohol containing an appreciable amount of water which might cause discoloration. This can be done readily by adding a pinch of white (anhydrous) copper sulfate to a small test quantity of the presumably water-free alcohol, and observing the behavior of the sulfate. If no change occurs in the color of the sulfate, the alcohol may be assumed to be practically free of water; but if the sulfate changes to a blue or a bluish green color, it means that enough water is present to make the alcohol unfit for use in the final dehydration stage. Alcohol that has absorbed too much water to be suitable for use in a given stage, may still be used if desired for a preceding stage by diluting it further with water to bring its concentration to the required lower level. The percentage of water in the alcohol can be determined conveniently by measuring the specific gravity of the liquid with a hydrometer.

Various types of nonrigid, durably pigmented specimens that are very soft or that contain a considerable amount of water, should be subjected to a preliminary preservation or hardening treatment before passing them through the alcohol dehydration stages. Such specimen material can usually be prepared by treating it with some one of the routinely employed preservatives or fixatives for biological specimens. The particular treatment to be used for a given specimen can best be determined experimentally, and in some instances it may even be advisable to carry through a trial embedment before deciding upon its suitability for the material in question. Furthermore it must be borne in mind that occasionally specimens may be encountered that do not lend themselves to preparation by any of the procedures in general use, and that embedment of these is not feasible unless special measures for satisfactory preservation are devised. Acceptable results may also be obtained in some cases by allowing the original colors to be destroyed by the preservation treatment, and then, after bleaching or clearing the specimens, staining them as desired.

A number of commonly used formulas that are suitable for the preservation and hardening of soft-bodied specimens are given below. In applying them it is important to allow ample time in every case for complete preservation, and to make sure that all traces of the treating agents are removed by washing in water or dilute alcohol before passing the material through the dehydration stages. For more detailed directions covering the routine preparation of biological specimens, textbooks or reference works on zoology or physiology should be consulted.

The following routine preserving fluids and fixatives may be found useful in preparing various types of biological specimens for embedment

in methacrylate plastics. All the compounds listed are poisonous, and some are flammable. Dry picric acid is highly explosive, especially in contact with metals or metallic oxides. Since some insurance policies contain a clause to the effect that the storage of high explosives in any quantity voids the policy, the purchase of picric acid, except in solution and in small quantity, is not recommended.

1. Formalin.—This is an aqueous solution of approximately 40 percent of formaldehyde gas in water to which a small amount of methyl alcohol has been added. It has good preserving action in most cases where the tissues are readily permeable, and it serves to toughen tissues and prevent shrinkage and distortion. It has but slight tendency to alter dark colors if removed completely from the specimen as soon as preservation has been accomplished. For most applications, formalin should be diluted with water to about 5 per cent of its original strength, although in some cases a concentration as low as 2 percent is adequate, while in others one as high as 10 percent may prove to be necessary.

2. Alcohol.—Ethyl alcohol is generally used for preservation and dehydration purposes. If tax-free laboratory alcohol is not available, isopropyl alcohol will be found to be almost equally suitable. Denatured alcohol may also be used if its formula does not include denaturants that are immiscible with water or otherwise unsuitable for application to specimen material. When used for preserving nonrigid material, alcohol should be diluted with water to 70 percent, or in the case of very soft material to 50 percent, of its original strength, to avoid unduly rapid dehydration and consequent shrinkage of the tissues. As soon as preservation is complete, the specimen material should be passed through the dehydration stages employing successively higher concentrations as quickly as practicable, because alcohol has a pronounced decolorizing action except in the water-free state.

3. Acetic acid.—Pure, or glacial, acetic acid tends to dissolve or alter the pigmentation and otherwise alter most types of biological material. It should be used therefore only in concentrations of 30 percent or lower, and preferably in combination with other preservatives. It serves to soften most tissues and in some cases acts as a clearing agent to render specimens translucent. Care should be exercised in handling acetic acid, because in concentrated form it can cause persistent burns.

4. Chromic acid.—Chromic acid (chromium trioxide) is an effective hardening agent for soft or gelatinous specimen material. Because of its tendency to oxidize and discolor specimens, its use is limited to special cases, and it should be applied for as short a time as possible for obtaining the desired result. It is used only in concentrations of 1 percent or less, and preferably in combination with other preserving substances. After being treated with fluids containing chromic acid, specimens should be washed with extra care to remove all traces of it.

5. Mercuric chloride.—Since mercuric chloride (corrosive sublimate) is violently poisonous, great care should be exercised when using it. It penetrates tissues rapidly and in many cases does not affect the color undesirably if removed quickly and thoroughly by washing as soon as proper preservation and hardening have been attained. It must always be completely removed before the specimen is dehydrated because it tends to be reduced to metallic mercury by alcohol with consequent blackening of the tissues. Mercuric chloride is generally used as a

saturated aqueous solution and in combination with another preserving agent such as acetic, chromic, or picric acid.

6. Formol-alcohol.—This frequently used preserving fluid can be prepared by adding 10 parts of formalin to 90 parts of 50-percent alcohol. Where desired 70-percent alcohol may be used instead of the 50-percent.

7. Alcohol-acetic acid.—(Carnoy's Fluid 3:1) Mix 95-percent alcohol, 3 parts, with glacial acetic acid, 1 part. The mixture may be diluted with water as required for various types of specimens.

8. Formalin-alcohol-acetic acid.—One mixture (Dietrich's or Kahle's Fluid) comprises: 95-percent alcohol, 30 cc.; formalin, 10 or 12 cc.; glacial acetic acid, 2 cc.; distilled water, 60 cc. A second mixture (Lavodowsky's Fluid) comprises: 95-percent alcohol, 50 cc.; formalin, 10 cc.; glacial acetic acid, 2 cc.; and distilled water, 40 cc. A third mixture (Wells' Fluid) comprises: 50-percent alcohol, 100 cc.; formalin, 6.5 cc.; and glacial acetic acid, 2.5 cc.

9. Chrome-acetic acid.—According to Wells, two mixtures are used. One comprises 1-percent chromic acid solution, 100 cc., and glacial acetic acid, 5 cc. The other comprises 1-percent chromic acid solution, 100 cc., and glacial acetic acid, 10 cc.

10. Chrome-acetic acid-formalin.—Mix 16 parts of 1-percent aqueous solution of chromic acid with 1 part of glacial acetic acid. Just before using, add one volume of formalin to two volumes of this solution.

11. Alcohol-chloroform-acetic acid.—(Carnoy's Fluid 6-3-1).—Mix 6 parts of 95-percent alcohol, 3 parts of chloroform, and 1 part of glacial acetic acid.

12. Carnoy-LeBrun Fluid.—Saturate a mixture of equal parts of absolute alcohol, glacial acetic acid, and chloroform with mercuric chloride.

13. Kleinenberg's Fluid.—Add 98 volumes of a saturated aqueous solution of picric acid and 2 volumes of concentrated sulfuric acid to 200 volumes of water.

14. Bouin's Fluid.—Mix 75 cc. of a saturated aqueous solution of picric acid with 25 cc. of formalin and 4 cc. of glacial acetic acid.

15. Rabl's Fluid.—Add 1 volume of a saturated aqueous solution of picric acid and 1 volume of a saturated aqueous solution of mercuric chloride to 2 volumes of distilled water.

16. Rath's Fluid.—Mix 1 volume of saturated aqueous solution of picric acid, 1 volume of hot saturated aqueous solution of mercuric chloride, and from one-half to 1 volume of glacial acetic acid.

17. Van Leeuwen's Fluid.—Mix 24 cc. of 1-percent solution of picric acid in absolute alcohol, 4 cc. of chloroform, and 4 cc. of formalin. Before using, add 2 cc. of glacial acetic acid.

18. Schaudinn's Fluid.—Mix 2 parts of a saturated aqueous solution of mercuric chloride with 1 part of 95-percent alcohol.

19. Worcester's Fluid.—Prepare a fresh saturated solution of mercuric chloride in 10-percent formalin. If desired, 9 parts of this solution may be mixed with 1 part of glacial acetic acid.

20. Zenker's Fluid.—Add 2.5 gm. potassium dichromate, 5 gm. mercuric chloride, 1 gm. sodium sulfate, and 5 cc. glacial acetic acid to 100 cc. water. The acetic acid should be added just before use.

In addition to treating specimen material with preserving fluids or fixatives, it may be found desirable in some cases to increase their

rigidity, after dehydration has been carried out in a liquid medium, by immersing them for several days in xylene (xylol) or toluene (toluol). In transferring specimens from absolute alcohol to xylene, and also from xylene to methacrylate monomer, three or more stages should be employed to insure proper elimination of the preceding liquid. It is also advisable not to permit soft or fragile specimens to dry out by evaporation during transfer, because they are liable to shrink or become distorted.

Caterpillars and other soft-bodied insect larvae, as well as certain worms and various other zoological specimens that have nonrigid integuments, can in some cases be prepared in an acceptable manner by killing them in water heated to approximately 60° C. (140° F.) and then gradually bringing the temperature to the boiling point and holding it there for 10 minutes, or longer if necessary, to coagulate the proteins and produce the desired toughening of the tissues. It is generally necessary to dehydrate specimens thus prepared by transferring them in the usual manner through several concentrations of alcohol, beginning with 70-percent and ending with 100-percent (absolute). These dehydrated specimens should finally be hardened by several days' immersion in xylene as previously explained.

Caterpillars and similar specimens can also be prepared, if preferred, by inflating and hardening them after removal of the body contents (*15, 16*). The method for doing this consists in laying the caterpillar on a sheet of paper and forcing out the contents by gently rolling a pencil or glass rod lengthwise along it, starting just back of the head. A glass tube drawn to a fine tip is then inserted in the anus, and the caterpillar is inflated to its natural size by carefully blowing into it. The distended specimen, with the tube still inserted, is finally hardened and dried by heating it cautiously over a low flame or in a moderately hot oven. Specimens prepared in this manner should preferably be stored in a desiccator to keep them dry until ready for impregnation and embedment.

Preparation of certain types of specimens that are difficult to process by any of the preceding methods may also be undertaken, if facilities permit, by means of the freeze-drying procedure outlined for processing unstable nonrigid specimen material in the next section (p. 54). However extensive equipment and special training are required for carrying out this procedure satisfactorily.

The second type of nonrigid durably pigmented specimen material is represented by flowers, leaves, and other plant parts that consist largely of thin tissues and contain comparatively small amounts of moisture. Generally specimens of this type having moderately stable pigmentation require no preliminary preservation and can be prepared for embedment simply by dehydration, followed if necessary by application of protective coatings. It should be borne in mind, however, that in this group many specimens may be encountered with which acceptable results cannot be obtained with any of the preparatory methods thus far employed. Such specimens must be considered unsuitable for embedment in methacrylate resins unless special procedures can be devised for them. Because of this uncertainty, it is always advisable in the case of flowers and similar material to try out preparation and embedment procedures experimentally with test material before attempting to work on the actual specimens.

Sometimes, in preparing specimens that consist mainly of thin tissues, removal of natural moisture can be carried out advantageously by dehydrating them in alcohol or similar water-absorbing volatile liquids, superior results usually being obtained with tertiary butyl alcohol. This should be carried out through several concentrations, starting with 40- or 50-percent and ending with the 100-percent or water-free liquid, in the manner described in the preceding section. The specimen material should be transferred from one concentration to the next as soon as it becomes uniformly infiltrated with the dehydrating liquid because there is an active tendency toward pigment extraction and discoloration where any appreciable amount of water is present in the dehydrating liquid. It is furthermore inadvisable to leave the material in the final dehydrating stage for more than a day or two before proceeding with the impregnation or coating step.

As soon as practicable after dehydration has been completed in this manner, specimens that are suitable for impregnation should be transferred to methacrylate monomer, or partial polymer, and impregnation and embedment carried out as described in the sections that follow. In the case of specimen material that shows a tendency to shrink or collapse when removed from the liquid, transfer should be made directly into the monomer through several successive baths as explained in the preceding section. Specimens that have sufficient dimensional stability to permit removal of the dehydrating liquid by evaporation, and also those that require protective coatings before embedment, should be freed of the dehydrating agent by placing them in a vacuum desiccator or in a moderately warm oven, held between 60° and 75° C. (140° and 167° F.) until all traces of the volatile liquid are gone. They should then be stored in a desiccator or other dry container until the impregnation or coating step is undertaken. In working with material dehydrated in this manner, it should be borne in mind that it is usually very brittle and requires considerable care in handling to avoid breakage. It is generally good practice to use forceps or tweezers for this purpose, and each specimen should be kept in a separate box or compartment during storage.

Various flowers and leaves, together with certain other objects composed of thin tissues, can be dehydrated satisfactorily without undue change in shape or color by packing them in a suitable granular or pulverized medium which absorbs moisture or permits its withdrawal from the specimen. In this method of dehydration, the packing medium employed may be a desiccating substance such as activated silica gel or various ground cereals that have been previously heated for several hours at 80° C. (176° F.); or it may be a medium that permits moisture vapor to pass through it, such as sand, talc, starch, sugar, borax, boric acid, salicylic acid, and powdered alum. The use of borax, boric acid, alum, plaster-of-Paris, talc, silica gel, cornstarch, rice flour, and various other substances for maintaining the natural appearance of flowers and leaves during dehydration was reported by Williams *(21, 22, 23)*. Sand or pulverized rock is reported to have been used for this purpose for several centuries.

Only well-dried, clean packing substances should be used in this procedure, and it is necessary to apply them with special care in order to maintain the natural shape of the dried specimens. Pouring the sand or other packing medium through a long-stemmed funnel has been found to

be a convenient way of directing its flow and building up proper support around the specimens. A cardboard box or other suitably porous container should be used in order to let the moisture escape readily, and it is advisable to perforate the bottom or hinge the sides so that the packing medium can be disposed of readily, after dehydration has been completed, without damaging the dried specimens buried in it.

In carrying out this dehydration procedure, the perforated box should just be set in a slightly larger one that has entire sides and bottom and that serves to prevent undue escape of sand from the inner box. After pouring approximately 1 inch of dry clean sand or other suitable granular or pulverized material into the box, place the specimens in position upon it. Then apply additional sand carefully, preferably through a funnel, in such a manner as to cover the specimens completely without disturbing the position of any of their parts. The specimens should be buried at least an inch below the surface, and if they have a tendency to curl or twist during drying, or if light-weight packing material is employed, it is advisable to pour on an additional 1 or 2 inches.

Specimens packed in sand or other nonabsorbent medium should be kept in the open air at a temperature about 10° C. (18° F.) above room temperature for 2 or 3 days. When silica gel or other activated absorbent is used, it is best to enclose the box in an airtight case containing a small amount of anhydrous calcium sulfate or calcium chloride to aid in absorbing the moisture.

At the end of the drying period the box containing the specimens should be raised from its outer container and the sand or other packing medium allowed to drain out. The dried specimens should then be carefully taken out, preferably with forceps or tweezers, and dust or particles of packing medium clinging to the surface should be removed with a soft brush or a feather, or in some cases with a gentle jet of air. If no compressed air source is available, blowing through a glass tube or straw will give satisfactory results if not continued long enough for moisture in the breath to dampen the specimen unduly. In the case of very fragile or brittle material, however, it is usually advisable to let it absorb a slight amount of moisture from the breath or from the air in the room in order to reduce its brittleness before attempting to clean away the adherent particles. After being cleaned, the dehydrated specimens must be kept dry by storing them in a desiccator or other suitable airtight container until they are to be coated or impregnated.

In the case of specimens that cannot be prepared satisfactorily by packing in a granular moisture-permeable or moisture-absorbing medium, good results sometimes can be obtained, if suitable equipment is available, by the freeze-drying procedure substantially as outlined for nonrigid unstable specimens in the next section.

Nonrigid material with unstable pigmentation.—This class of specimen material includes body tissue together with various types of zoological and botanical specimens that require special preparation to retain both shape and color. Considerable experimentation is sometimes required for determining which of the known procedures is best, or for working out special procedures, for preparing such material; and, furthermore, numerous specimens may be encountered that prove to be altogether unsuitable for embedment in methacrylate resins.

For the preparation of body tissue and organs, as well as certain

fleshy animal or plant specimens, generally satisfactory results can be obtained, when adequate facilities are available, through the use of a special freeze-drying procedure. The preparation of tissue specimens by freeze-drying and their subsequent embedment in methacrylate plastic blocks was described by Strumia and Hershey in 1944 (*19*); and the equipment used in connection with this work was described by Strumia and McGraw (*20*). The method [4] employed for preparing specimen material in this manner consists of first wetting the specimen thoroughly and then placing it on a base of ice in a metal pan and freezing it in a low-temperature cabinet at between —20° and —25° C. (—4° and —13° F.). As soon as the specimen is frozen, sufficient water is put in the pan to form a layer approximately one-fourth inch deep. After this has frozen, successive layers are added and frozen until the specimen is embedded in a block of ice that covers it to a depth of at least one-fourth inch. It has been found advisable to glaze large specimens by spraying them with an atomizer during the initial freezing before embedment in the successively frozen layers of ice. Material prepared in this way should be kept in the frozen state until transferred to the drying apparatus, and, if desired, it can be stored in this condition for many months without apparent change.

Dehydration is carried out in a vacuum system of the type employed for drying biologicals from the frozen state, which must be capable of maintaining a temperature of approximately —15° C. (5° F.) in the specimens while the ice in them is being removed by sublimation. During dehydration, the ice blocks containing the specimens are kept in loose gauze bags held in wire baskets, and are subjected to continuous drying action until all moisture has been removed. This usually takes from 5 to 15 days, depending upon the size of the specimens.

After being dehydrated, the specimens should be trimmed with a sharp knife to remove all loose portions and then kept in completely dry condition in a desiccator until the time for impregnation and embedment. Best results are insured by carrying out these final steps within a few days, although it is usually possible to store the dry specimens for several weeks without appreciable deterioration. While in the dry state, specimen material prepared in this way appears pale and discolored but much of its original color is restored as soon as the specimen is impregnated with methacrylate monomer or partial polymer.

In certain cases acceptable results with animal and human tissue specimens can be obtained by processing them with the Kaiserling or the Klotz fluids (*4, 9*), or with suitable modifications of them, and then dehydrating them in the usual manner, beginning with 70-percent and ending with 100-percent (absolute) alcohol or other suitable volatile water-removing agent The monomer impregnating and embedding operations should be perfomed promptly upon completion of dehydration, and the polymerization of the plastic during embedment should be carried out at the lowest elevated temperature practicable in order to avoid undue alteration of the original color.

The preparation of tissue specimens by a modification of the Kaiserling method, which has been found suitable for this application, is described

[4] The procedure to be followed in applying this method is explained in detail in the instruction sheets on embedding biological specimens in plastic, issued by the Rohm and Haas Co., Philadelphia, Pa., Aug. 1947.

in detail by Bengston (*4*). Color preservation by combination of Klotz and Kaiserling methods was explained by Aegerter (*3*). Application procedures for the Kaiserling and the Klotz methods, together with modifications of these methods, are described by various authors including Galt (*7*), Pearl (*17*), and Judah (*9*).

Specimens of either plant or animal material that do not lend themselves readily to preservation by any of the foregoing methods may in some instances yield acceptable results if they are treated with polyhydric alcohol ester preserving fluids and then processed in the manner explained under Procedure C in Part 1 (p. 19). The basic process for thus preserving the natural color in plant specimens as described therein is, however covered by a patent in which the U. S. Government has permitted the patentee to retain commercial rights. Consequently, this process cannot be used for profit-making purposes; but the patentee has given his consent to the use of his basic process, as well as the modifications thereof, described in Part 1 of this bulletin, for educational and other nonprofit purposes.

Specimen material preserved by the special process just referred to does not as a rule retain its original shape, and for this reason its embedment ordinarily has to be carried out in more or less two-dimensional form. The use of this special method is, therefore, to be considered only in cases where it is desirable to have the natural color of the specimen retained at the sacrifice of this shape. In most other cases it will be found preferable to prepare the material by one of the foregoing procedures which will permit retention of the three-dimensional form at the expense of the natural color.

Impregnating Specimens With Monomeric Methacrylate

Except in the case of nonporous material and material that has been coated to protect it from direct contact with the plastic, specimens should preferably be impregnated with the monomeric liquid methacrylate to eliminate impounded air and to insure suitable penetration of the partial-polymer sirup during embedment. It is important to make sure that all specimen material is dry before being placed in the monomer for impregnation, because moisture, if present in appreciable amount, may be carried into the plastic block and cause cloudiness. As previously explained also, it is well to employ enough intermediate immersions, when transferring specimens from alcohol or other volatile liquids, to make certain that practically all of the dehydrating agent is eliminated and thus avoid the possibility of undesirable vapor bubbles being formed in the block as polymerization progresses.

Impregnation of specimens should always be carried out under a fume hood, and all vessels containing the monomer should be kept closed as much of the time as possible. (See cautions under "Equipment and Supplies," p. 32 et seq.) Glass containers, covered with suitable glass or metal plates or with tinfoil or cellophane, should be employed so the specimens can be watched easily during impregnation. Only clean, inhibitor-free, uncatalyzed monomer should be used. When it becomes discolored or dirty through repeated re-use, it should be discarded unless its use as a preliminary washing or extracting medium is desired.

Certain insects and other specimens that have more or less impervious

integuments should be punctured in two or more places with a dissecting needle or other sharp implement before immersion to facilitate entrance of the monomer into the body cavities. In the case of large specimens it might also be advisable to inject monomer, or partial polymer sirup, into the principal cavities through the use of a syringe.

Fragile specimens, and also those that are already permeated with monomer as the result of being transferred from a liquid dehydrating medium, should be allowed to stand completely covered with monomer for at least a day, and during this time it is best to place the vessels containing the specimens in a refrigerator where a temperature of about 5° C. (41° F.) is maintained to guard against premature polymerization. If desired, most specimens may be kept for a considerable length of time in monomer, provided they are stored at a temperature low enough to retard polymerization. Specimens that contain extractable pigments, however, should be removed from the monomer bath as soon as full saturation has been attained and embedded as promptly as possible by means of the quick-setting, viscous, partial-polymer sirup.

Specimens that are porous or of a generally permeable nature can usually be impregnated to the best advantage under partial vacuum, if they are sufficiently strong to withstand the effect of the escaping air bubbles. Such specimens should be immersed in monomer, or in some cases in thin partial-polymer sirup, and the container, after being loosely covered with a flat plate of glass or metal, should be placed in a vacuum desiccator. An aspirator rather than an oil-type vacuum pump should be used to evacuate the desiccator, because the methacrylate vapor would tend to dilute the oil in the latter and eventually impair its operation.

A suitable trap should be interposed between the aspirator and the vacuum chamber to serve as a safeguard against entrance of water in case of a temporary reduction of pressure in the water-service lines during evacuation. A vacuum gauge or manometer in the system is also desirable so that the degree of vacuum can be readily noted at all times. It is always best to keep the vacuum-chamber stopcock closed until after the water has been turned on full force through the aspirator, and likewise to close the stopcock before the water is shut off.

In subjecting the specimen to vacuum, a negative pressure of between 25 and 29 inches of mercury will usually be found to be suitable. It is important, however, to watch the specimen closely and to control the vacuum so that violent bubbling is avoided. The removal of entrapped air is usually facilitated by alternately evacuating the chamber and then allowing air to return into it so as to produce a pumping action on the specimen. It is advisable to continue evacuation in this manner until no more air bubbles are given off, or at least until the specimen tends to sink into the monomer under normal atmospheric pressure. Whenever air is allowed to re-enter the chamber it should be admitted slowly in order to avoid strains which might cause breakage of the glass. It is also advisable to watch the monomer for possible thickening due to premature polymerization, and to replace it with new monomer before it becomes unworkably viscous.

As soon as the specimen has become completely saturated with monomer, it is ready for embedment. If the embedding operation is not to be carried out at once, the specimen should be kept immersed in the liquid methacrylate and stored in a cold place.

CASTING SUPPORTING BASES FOR SPECIMENS

In order to support the specimen in the center of the finished block, a preliminary layer of plastic must first be cast in a suitable mold (fig. 13). The best results in casting methacrylate resins are usually obtained with glass molds because they permit watching the specimen as polymerization progresses and also easy removal of the block when it has hardened. A mold should be chosen that will yield a cast block of the desired shape and size and is large enough to provide for surrounding the specimen with at least 1/4 inch of plastic on all sides.

For making cylindrical castings, Pyrex-type glass beakers are con-

FIGURE 13.—A small amount of casting sirup is first poured into the mold and polymerized to serve as a supporting base for the specimen.

venient, although glass jars or tumblers might prove to be more economical in most instances. Electric light bulbs with the neck portion cut off can be used for making globular castings. Molds for rectangular castings can be made by binding together glass plates of the proper size with suitable adhesive tape on the outer edges and then sealing the seams on the inside with gelatin, casein, egg white, or polyvinyl alcohol. A 10-percent solution of gelatin, plasticized with 5 to 10 percent of glycerin, sorbitol, or polyethylene glycol and applied while warm with a small round brush will be found to be suitable for this purpose. The gelatin or other sealing substance should be thoroughly dried by baking in a moderately warm oven before the mold is used. Glass refrigerator dishes are serviceable for larger specimens, and in some cases it may be found convenient to embed several specimens in one shallow block and then saw the casting into separate mounts after hardening.

Molds made of plaster of Paris, or of wood or cardboard, may also be employed if their inner surfaces are coated with gelatin or other suitable material to prevent absorption of monomer and facilitate release of the hardened casting. Gelatin capsules have been used as molds for embedment of very small specimens.

To cast the supporting base for the specimen, pour into a dry, clean mold a layer, up to one-half inch in thickness, of the most viscous grade of partial-polymer casting sirup that has been prepared. In deciding upon the thickness of this supporting layer, it should be borne in mind that a shrinkage of approximately 20 percent will occur during hardening, together with an additional loss of from 2 to 5 percent through uncontrollable volatilization. It is also necessary to allow for the possible removal of 0.1 inch or more from the bottom of the block by sanding and polishing when finishing it.

Pouring the casting sirup for the base layer, and for the subsequent embedding layer as well, should always be done in a fume hood or other enclosure from which the air is continuously withdrawn to guard against diffusion of the escaping vapor into the room. For the pouring operation it will usually be found convenient to place the mold on an elevated stand or platform so that it will be nearly level with the eyes and thus facilitate judging the true depth of the sirup in it. When working with the more viscous grades of partial polymer, it is necessary to use extra care in controlling the flow in order to avoid pouring too much into the mold. This can be done by gradually tilting back the flask as the sirup begins to approach the desired level in the mold, and finally "cutting off" any surplus sirup with a glass rod and returning it to the flask.

The mold into which the base layer of partial polymer sirup has been poured must be well covered with tinfoil or cellophane suitably pressed down or tied in place so as to prevent escape of vapor as effectively as possible during polymerization. In the case of large castings, the covered mold should be allowed to stand for an hour or two, preferably in the refrigerator, before it is heated in order to permit all impounded air to rise to the surface and escape. This step may be omitted with small castings, however, provided they are inspected two or three times during the first hour in the oven and are transferred promptly to the refrigerator if air bubbles appear to be trapped in the semifluid plastic. In this case, elimination of bubbles can usually be facilitated by pouring a small amount of unthickened monomer on the surface of the viscous partial

polymer and then probing into the bubbles with a clean dissecting needle or a small glass rod to allow the air to escape.

To bring about polymerization and hardening of the base layer of plastic, the mold, with its covering in position, should be placed in a constant-temperature oven heated by hot water or electricity. By no means should gas or oil-burner ovens be employed; and if electric ovens are used, only those types with external thermostat-control contacts and explosion-proof doors that cannot be positively latched should be considered safe for this purpose. The temperature should be maintained at about 45° C. (113° F.) if methyl methacrylate sirup is being used, or 50° C. (122° F.) in the case of ethyl methacrylate, until the base layer has become polymerized to a firm gel consistency, which usually requires from 40 to 50 hours. The temperature should then be raised 5° C., either by changing the thermostat setting or, preferably, by transferring the mold to a second oven that is regulated to maintain a temperature of 50° C. (122° F.) for methyl methacrylate or 55° C. (131° F.) for ethyl methacrylate. Slightly higher temperatures may be used for small castings, but in all cases care is necessary in heating the plastic while in the gel stage to keep the temperature below the point that will cause vapor bubbles to form and remain trapped in it.

As a means of testing the hardness of the plastic as polymerization progresses, a sharp-pointed implement such as a dissecting needle or a scalpel should be pressed gently into it from time to time while it is in the oven. When the upper surface finally becomes so hard that it is no longer dented to a noticeable extent by the testing implement, it may be considered that polymerization has progressed far enough and the base is ready for the embedding operation. A convenient guide in determining the progress of polymerization as the plastic changes from gel to solid state, is the temporary line or plane that becomes visible between the gel and the denser solid portion below and moves gradually upward as the hardening proceeds. When this line reaches the top and disappears it indicates that the required degree of hardness has been attained throughout the block.

As soon as the base casting has become completely solid, the mold should be removed from the warmer oven and gradually cooled down to room temperature. This can be done by transferring it back to the cooler oven for a few minutes, taking it out and setting it for a short time on the slightly warm top of the oven, and finally placing it on a table or bench until it has completely cooled to room temperature. If it is not to be used at once for the embedding operation, it should be carefully protected from dust because otherwise a visible layer will be formed when the remainder of the plastic is added.

EMBEDMENT OF SPECIMENS

The embedding operation consists of placing the prepared specimen on the previously cast plastic base in the mold (fig. 14), then surrounding it with fluid partial polymer or with monomer, and finally hardening the entire mass through polymerization to form a solid block. In carrying out this procedure minor variations are required, or optional, for different types of specimens, depending upon their size and structural characteristics and upon the methods employed for their preparation.

Figure 14.—The impregnated specimen is removed from the monomer liquid with tweezers (*A*); it is then placed in position on the previously hardened base in the mold (*B*).

At the time of embedment all specimens must be sufficiently dry to insure no diffusion of moisture into the plastic, and, where dehydration has been carried out through the use of alcohol or other volatile liquids, no appreciable amount of the liquid dehydrating agent must remain in the specimen. If these precautions are not observed, the polymerized plastic is likely to be clouded in the first case or to contain permanent vapor bubbles in the second. Specimens that have not been impregnated with monomer or partial polymer prior to embedment, either because they have impervious surfaces or because they have been coated to protect them from direct contact with the fluid plastic, should preferably be kept in a desiccator until the embedding operation is started, although it is reasonably safe in most cases to store them in the open air if the relative humidity in the room is continuously lower than 30 percent.

The first step in the embedding operation is to pour a small amount of fluid plastic upon the base in the mold. Partial polymer of medium viscosity is generally preferable for this purpose, except where extreme porosity of the specimen material necessitates the use of unthickened monomer or where impervious or coated specimens call for the highly viscous grade. In the last case, where very viscous casting sirup is employed, it is usually advisable first to soften slightly the upper surface of the base by covering it for a minute or two with monomer, which may then be poured back into the stock container.

When casting methacrylate resins in block form, it is important to guard against excessive internal temperature rises, which tend to occur because of the exothermic nature of the polymerization reaction and the low heat conductivity of the solid polymer. Since temperatures high enough to damage both block and specimen are frequently produced in

this way during hardening of masses of fluid resin that are more than one-half inch thick, it has been found advisable to restrict the size of single-layer cast blocks to a point where this ½-inch limit is not exceeded. When larger blocks are desired, it is generally necessary to employ the layer-by-layer method described in a patent by Kuettel (*29*).[5] This calls for alternately pouring and hardening successive layers of fluid resin that do not exceed the ½-inch limit of thickness required for satisfactory polymerization. If this procedure is carefully carried out with due regard for the elimination of dust and other foreign matter between layers, blocks of practically any desired size that are clear and homogeneous and free from lines of demarcation between the layers can be produced.

In the embedment of objects that tend to float in the fluid resin, the first layer poured upon the base should be just thick enough to anchor the specimen in place after solidification. It is also advisable to restrict the next layer to a depth that will not tend to cause undue softening of the anchoring layer before polymerization can take place. The remaining layers may be of any convenient thickness up to the ½-inch limit. In the case of heavier specimens that sink in the fluid plastic, any desired depth up to the ½-inch limit may be employed for the first layer as well as for the subsequent ones. Successive layers should be added in this manner, making sure that each is adequately polymerized before the next is poured on, until the specimen is completely embedded and is covered to a sufficient depth to allow for machining and polishing the finished block.

To embed the specimen pour the first layer of casting fluid of requisite viscosity on the base to the proper depth indicated by the nature of the specimen material. Then introduce and orient the specimen, preferably with smooth-tipped forceps or tweezers or with dissecting needles. Extreme care is necessary in arranging fragile objects because they tend to adhere quickly to the base and are liable to be torn or broken if an attempt is made to move them afterwards. In the case of specimens that have hollows or cavities on the underside, it is usually advisable to mount them upside-down to facilitate the escape of trapped air, and then to reverse the block when finishing it after the casting has been completed.

As soon as the specimen has been placed in position, the mold should be effectively covered with tinfoil or cellophane to prevent undue vapor loss and then allowed to stand for an hour or two at room temperature to permit the trapped air to escape. If the bubbles do not rise properly or the fluid plastic does not enter the hollow spaces, it is advisable, except where very viscous casting sirup has been used, to place the mold in a vacuum chamber for a short time (fig. 15). In doing so, however, the process should be watched closely, and the degree of vacuum controlled so that excessive bubbling or frothing does not occur.

When the fluid plastic surrounding the specimen appears to be freed of air and the submerged part of the specimen is suitably impregnated, the mold, with its covering in place, should be set in a water-bath or in a constant temperature oven of about 45° C. (113° F.) in the case of methyl methacrylate, or 50° C. (122° F.) for ethyl methacrylate, until

[5] Patent under assignment to E. I. du Pont de Nemours & Co., Inc. The process covered by this patent cannot be used except by consent of the assignee.

FIGURE 15.—Bubbles in the casting sirup and entrapped air in the specimens are removed after filling the molds by placing them in a vacuum desiccator. The small quantity of vapor is removed from the closed desiccator by suction.

the embedding layer has become a firm gel. The temperature may then be increased to 50° C. (122° F.) or to 55° C. (131° F.), respectively, either by changing the thermostat setting or by transferring the mold to a second oven or water-bath regulated for operation at the higher temperature. Temperatures slightly higher than these may be employed safely for small castings, but they are not recommended for blocks much over 1 inch

in diameter or where the embedding layer is the full one-half inch in thickness.

Carrying out polymerization of masses of methacrylate resin under controlled temperature conditions that permit removal of exothermically produced heat, as well as provide the heat needed for initiating the reaction, was described in a patent by Loder (*30*)[6].

When the first embedding layer has become fully hardened, the mold should be removed from the oven. Determination of the extent to which polymerization has progressed can be made by testing the hardness of the plastic with a dissecting needle or other sharp-pointed implement. After careful cooling to avoid breakage as explained in the section on the casting of bases, the mount will be ready to receive the next layer. In applying this, as well as all remaining ones, the same procedure should be followed, and the same polymerization temperatures employed, as for the initial layer. The amount of time required for completing the embedment may be shortened somewhat in the preparation of small mounts of durable specimens by using thicker embedding layers and by raising the final polymerization temperatures by 5° or 10° C. (9° or 18° F.); but on the other hand when unstable or fragile specimen material is to be embedded, it will often be found worthwhile to proceed even more slowly, using thinner layers and lower temperatures, in order to insure more uniformly satisfactory results.

Annealing to Prevent Crazing

Unless special precautions are taken, the development of minute surface cracks or crazing is almost certain to occur within a few months in blocks of methacrylate plastic cast in the manner here described. This undesirable condition can be prevented, however, if the cast blocks are annealed or heat-treated before removal from their molds. Prevention of crazing in cast methacrylate polymer by prolonged heating at suitable elevated temperatures was described in a patent by Kuettel (*28*)[7]. The procedure for this treatment consists in subjecting the polymerized plastic to prolonged heating at as high a temperature as the embedded objects can tolerate. In the case of durable specimen material capable of withstanding at least 75° C. (167° F.), heating at this temperature for from 10 to 20 hours, dependent upon the size of the block, is usually sufficient to eliminate all likelihood of crazing. With blocks containing specimens that are not stable at temperatures much above 50° C. (122° F.), heating for 3 weeks at 50° C. is generally necessary. If they can tolerate 55° C. (131° F.) heating at that temperature for 2 weeks should be sufficient. Specimen mounts having a maximum temperature toleration between 55° and 75° C. call for heat-treating periods of intermediate length.

Upon completion of the heat-treating cycle, the casting should be cooled cautiously to avoid breakage of the mold from uneven contraction. This can be done conveniently in most cases by setting the mold with its casting in a vessel of water that has been heated to about the same temperature as the casting and allowing it to cool gradually to room temperature.

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[6] Patent under assignment to the du Pont Viscoloid Company, and cannot be employed except by consent of the assignee.

[7] See footnote 5, page 62.

Removal of Cast Blocks From Their Molds

After the cast block in which the specimen is embedded has become entirely cool, it can sometimes be removed from its mold by shaking or tapping the open end of the mold on a smooth firm surface. In cases where a block cannot be removed in this manner, it should be loosened as much as possible by running a scalpel or strong-bladed knife around its rim to free it from the wall of the mold, after which the shaking or tapping should be repeated. If this fails to bring it out, the mold should be filled with water and set aside for a day or two. After the mold is emptied and dried at the end of this time, the cast block will usually be found to be free enough to permit easy removal. Where a Pyrex-type glass mold has been employed, easy release of the casting can often be accomplished by first chilling the mold and block to a low temperature in the freezing compartment of a refrigerator, and then quickly pouring hot water over the outside of the mold to expand it while the block is still in a cold contracted state.

Satisfactory aid in removal of castings may also be obtained in some cases by coating the inner walls of the mold with a suitable releasing agent before pouring in the liquid plastic. Only such substances should be used for this purpose, however, as are insoluble or not readily miscible in the liquid resin and have no detrimental or discoloring effect on the polymerized plastic. Thoroughly dried coatings of soap or of certain types of surface active agents have proved to be suitable in some instances, as have also various commercially available mold-release compounds primarily intended for use in connection with pressure molding of plastics.

In any case where none of the foregoing methods yields the desired result, it will be necessary to break the mold away from the casting. In doing so it is advisable to protect the hands with gloves and to use tweezers or forceps to pick off all glass splinters adhering to the casting. The mold should be broken by striking it with a hammer or squeezing it cautiously between the jaws of a vice in a manner that will not mar or otherwise damage the cast block.

FINISHING THE CASTINGS

Upon removal from their molds, castings in which specimens have been embedded ordinarily require a limited amount of finishing to take care of surface irregularities and render all their faces uniformly smooth and clear. The finishing procedure consists mainly of machining, sanding, and polishing operations that are carried out much as in the case of brass or hardwood, and may be performed with the tools and equipment generally used for work on such materials.

Detailed directions that are applicable to work on methacrylate castings may be found in manuals [8] issued by producers of acrylic plastics, as well as in various texts dealing with mechanical work on plastics in general. For the benefit of those not having access to such publications,

[8] Among the publications that contain useful information in this connection are: *Lucite Methyl Methacrylate Resin Manual*, issued by Plastics Department, E. I. du Pont de Nemours & Co., Inc., Arlington, N. J., Nov. 1942 (and subsequent dates); and *Plexiglas Fabricating Manual*, issued by Rohm and Haas Company, Philadelphia, Pa., Nov. 1941 (and subsequent dates).

the principal finishing operations applicable to methacrylate plastic castings are given here in outline based on the data furnished in certain fabricating manuals.

Machining

Minor irregularities can in most cases be taken care of by leveling them off with a smooth-cut file or by applying coarse- or medium-grit sandpaper. When larger sections are to be removed it is more satisfactory to cut them off with a saw. Saws of almost any type may be used—either hand or power operated—but better results are usually obtained with one that has 8 or more teeth per inch with little or no set. In sawing, it is important to avoid too great pressure, which might result in overheating and gumming, and to keep the saw-cut straight so that heat from undue side pressure will not cause binding or sticking.

Power-driven saws should be provided with suitable devices to protect the operator against personal injury and in using such saws caution must be exercised to avoid damaging the plastic in which the specimens are embedded.

It is generally necessary to use a lathe for truing the round surfaces of cylindrical castings. In such instances the sanding and polishing, which follow the machining operation, can be carried out conveniently on the blocks while they are still set up in the lathe. Either a wood-turning lathe or a metal-turning or engine lathe may be used for machining methacrylate castings, but the metal type is generally preferable because it permits more accurate work.

Coolants may be used if desired to aid in eliminating excessive frictional heating in the various machining operations required in trimming and surfacing the plastic castings. No fluids that contain solvents or other detrimental ingredients should be used, and in most cases soapy water, or even plain water, will be found to be satisfactorily effective.

Sanding

After all the faces of the block requiring resurfacing have been properly trued, they should be sanded or ashed until they are free from noticeable scratches and depressions. For the sanding process it is best to use wet-or-dry type of sandpaper, and to apply ample water during the several stages of sanding to minimize scratching from loose grit.

It is usually advisable to start with moderately coarse paper (240 grit or equivalent) and then change to successively finer paper as the coarser scratches are sanded out. Intermediate grits of 320 and of 400 should thus be used, followed finally by 500 or preferably 600, until the work has a satiny surface free from visible scratches. This operation may be carried out entirely on a power driven sander if desired, but the most satisfactory results are to be obtained by applying the final stages by hand. In all hand sanding it is important to have the sandpaper laid out on a practically true surface and held securely in position to prevent slippage. A sheet of flat pressed window glass, or preferably plate glass, is very good for this purpose, and springy strips of metal attached to a wooden base supporting the glass plate can be made to serve as clamps for holding the sandpaper in place.

In resurfacing cylindrical blocks, it is usually more convenient to employ ashing with wet pumice applied on a power-driven cloth buffing wheel than to use sandpaper. Such procedure may also be employed for rectangular blocks if desired, but it is inadvisable in most cases because it tends to curve the surface and round the edges excessively.

After the sanding and ashing operations are completed, the block should be well washed with clean water to remove all grit and dust before polishing is undertaken.

In blocks that are in acceptably good condtion when first removed from the mold, minor scratches or irregularities can be taken care of by the final buffing and waxing without the need of any preliminary sanding.

Polishing

Properly surfaced methacrylate castings can readily be given a clear lustrous finish by polishing with suitable abrasive compounds on power driven buffing wheels. For this purpose felt wheels are considered to be the most satisfactory, but cloth wheels can be made to give acceptable results if properly used. These buffing wheels may be from 8 inches to 15 inches in diameter and should preferably be built up to a thickness of approximately 3 inches. They may be operated at surface speeds of between 1,200 and 1,800 feet per minute, but extra care is needed at the higher speeds to avoid undue pressure of the work against them which might cause excessive frictional heating with consequent scarring or "burning" of the surface of the plastic. In this connection it should also be borne in mind that localized overheating during finishing is one of the main causes for crazing or superficial cracking at a later time.

Among the satisfactory abrasives for polishing methacrylate plastics, magnesium carbonate and also prepared calcium carbonate (whiting) have been found to be particularly suitable. In using abrasives of this type, it is advisable first to touch a stick of tallow to the revolving wheel, and then to apply as much of the compound as will adhere to the wheel. A number of special polishing compounds for plastics are also commercially available, and those that are indicated as being suitable for use on acrylic plastics may be expected to give good results. Jewelers' rouge can be used if desired for this purpose, but it is recommended only where other more satisfactory compounds are not available.

When a uniformly smooth, clear surface has been obtained with the buffing wheel carrying the polishing compound, it is advisable to transfer the operation to a second wheel, preferably a cloth wheel of open type, which is free from abrasive. This will serve to clean the block and give it a final high luster.

If it is desired to maintain very true surfaces and sharp edges on the finished block, the polishing operation should be carried out on a large felt pad, preferably on a flat revolving disc. After moistening this polishing pad with water, apply a suitable amount of the abrasive compound and distribute it over the surface; and from time to time, as the polishing progresses, add more water and compound as needed. The plastic block should be held against the polishing pad with even and moderate pressure, and it should be moved over the surface at a comparatively

FIGURE 16.—Specimens prepared by C. E. Sando show the clarity and luster obtainable through accurate machining and polishing of the finished blocks.

slow rate. This method of polishing is time-consuming but it yields excellent results when properly carried out (fig. 16).

WAXING

The polished surfaces can be given a fair degree of protection against dirt and abrasion, and their maximum luster maintained for a longer period, if they are covered with a durable wax coating. Polishing waxes

of the emulsion-paste type used for automobiles are suitable for this coating, as are also a number of other commercially available nonfluid preparations that are specially offered for this purpose. Liquid waxes, on the other hand, and others containing active solvents or other detrimental ingredients, must be carefully avoided because of their tendencies to scratch the surface or cause crazing.

In applying this protective coating, the wax should be put on with a damp cloth, and then polished vigorously with a piece of clean flannel or other suitably soft woolen cloth until a high luster is obtained.

CARE OF FINISHED MOUNTS

Methacrylate plastic mounts are not affected by moisture or natural temperature variations, and light has practically no effect upon them except that it may alter the specimen colors when they have not been fully stabilized before embedment. The mounts are, therefore, very durable, and, if not subjected to careless handling or exposure to active solvents or solvent vapors, they may be expected to retain their original appearance almost indefinitely. They are somewhat easily scratched, however, and for that reason should be kept coated with a thin protective layer of wax and handled with reasonable care to avoid undue abrasion. It is furthermore advisable to cover them with protective wrappings or soft packing material when shipping or transporting them.

Cleaning

Whenever it becomes necessary to clean mounts of this type, it is best, if possble, to do so with clear water or with water to which pure soap has been added. Gritty soaps, as well as most types of synthetic detergents, should be avoided. Ammonia water, however, may be used if needed to remove grease and stains, and it is also safe to use kerosene or mineral spirits for this purpose. Practically all other solvents, as well as solvent cleaning mixtures not specifically designated for use on acrylic plastics, should consistently be avoided because of their tendency to induce crazing. In drying the blocks after cleaning, it is advisable to use soft tissue paper or clean, soft cloth or chamois skin. Similar materials should also be used when wiping off dust or nongritty dry dirt.

After cleaning, it is always good practice to apply a new coating of protective wax in the manner described in the preceding section.

Refinishing

In cases where blocks have become dull or scratched, they should first be cleaned with mineral spirits or kerosene to remove the wax coating, and then, if necessary, washed with soapy water. After this they can be resurfaced by wet-sanding according to the procedure previously described; they are then ready for polishing and waxing in the usual manner. Minor scratches and abrasions can usually be eliminated satisfactorily by the simple application of a new coating of wax followed by vigorous polishing with a soft flannel cloth.

Blocks in which crazing has developed can, in some cases, be satisfactorily reclaimed by sanding them down or otherwise resurfacing them

to a sufficient extent to eliminate the cracked areas, and then refinishing them as before. It is better practice, however, to place such blocks in fairly tight-fitting molds and subject them to the annealing heat-treatment for a suitable length of time before proceeding with the resurfacing and polishing.

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